

Standard Operating Procedure		Page 1 of 4
<b>Autoclaving of Potentially Infectious Wastes</b>		
Investigator: All	Location: All OSU Facilities	Revision: 00

**1.0 PURPOSE:**

The purpose of this SOP is to ensure that potentially infectious waste materials are adequately sterilized when subjected to autoclaving. State law requires that all autoclaves used for decontamination of infectious materials be posted with operating procedures and tested monthly for efficacy following the parameters of the operating procedures. Autoclaves used for waste treatment should be dedicated for that purpose.

**2.0 SCOPE:**

This SOP applies to all Oregon State University facilities in the state of Oregon where autoclaving is used to decontaminate infectious materials prior to release to the normal waste streams (sanitary sewer for liquids, permitted landfill for solid wastes). This document shall be posted in a visible location near each autoclave used to decontaminate infectious materials. Sterilization of wastes shall be conducted in accordance with the parameters defined in this document.

**3.0 RESPONSIBILITIES:**

All operators of autoclaves are responsible for operating the autoclave in accordance with the parameters defined in this document when that autoclave is being used to decontaminate potentially infectious regulated medical wastes. Operators are also responsible for running monthly tests in accordance with the instructions in the kits supplied by Environmental Health & Safety. Departmental or facility "owners" of autoclaves are responsible for maintaining autoclaves in good working order and having the autoclaves tested annually by a qualified technician. Environmental Health & Safety BSO is responsible for distributing autoclave test kits, incubating returned test vials, providing operators with a report of test results, and maintaining campus-wide record system for the testing program. Environmental Health & Safety BSO is responsible for providing training and guidance on effective decontamination methods to the research community as needed.

**4.0 DEFINITIONS:**

SOP = standard operating procedure

BSO = biological safety officer

"Potentially infectious wastes" needing to be autoclaved include the following:

- "Biological waste", which includes blood and blood products, excretions, exudates, secretions, suctionings and other body fluids that cannot be directly discarded into a municipal sewer system, and waste materials saturated with blood or body fluids, but does not include diapers soiled with urine or feces.
- "Cultures and stocks," which includes etiologic agents (of disease) and associated biologicals, including specimen cultures and dishes and devices used to transfer, inoculate and mix cultures, wastes from production of biologicals, and serums and discarded live and attenuated vaccines. "Cultures" does not include throat and urine cultures.
- Gloves and other disposable personal protective equipment used as barriers when handling biological wastes or cultures and stocks.

**5.0 REFERENCES:**

Originator: M. Philpott, OSU Biosafety	DOC #: A-1	Active Date: February, 2007	Retired Date:
Reviewed:		Approved:	
_____	_____	_____	_____
Name & Title	Date	Name & Title	Date

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Oregon Statutes, Chapter 763, enacted 1989

Oregon Department of Human Services, Public Health Chapter 333, Division 56, "Infectious Waste Management."

Rutala, W. A., Stiegel, M. M. and F. A. Sarubbi, Jr. *Decontamination of laboratory microbiological waste by steam sterilization.* App. Env. Microbiol. 43: 1311-1316 (1982).

Lauer, J. L., Battles, J. R. and D. Vesley. *Decontaminating infectious laboratory wastes by autoclaving.* App. Env. Microbiol. 44: 690-694 (1982).

Ozzane, G., Huot, R. and C. Montpetit. *Influence of packaging and processing conditions on decontamination of laboratory biomedical wastes by steam sterilization.* App. Env. Microbiol. 59: 4335-4337 (1993).

## 6.0 MATERIALS and/or EQUIPMENT:

Autoclavable bags for waste collection  
 Leak-proof secondary container with lid for placing autoclavable bags in during collection  
 Black plastic bags to over-pack treated waste bags  
 Shallow tub or tray, metal preferred but can be autoclavable plastic  
 Rack that fits within shallow tub or tray  
 Heat-sensitive autoclave indicator tape

## 7.0 PROCEDURES:

### 7.1 Waste Collection

- 7.1.1 All potentially infectious wastes (see definition above) in laboratories must be collected into autoclavable bags adorned with a biohazard symbol. Such wastes shall be segregated at the point of collection from ordinary wastes that are not potentially infectious (i.e., paper towels, supply wrappers, etc.)
- 7.1.2 Autoclavable bags used for the collection of potentially infectious wastes are to be placed within a leak-proof secondary container with a lid. These containers must be adorned with a biohazard symbol in red or orange. Commercially available waste receptacles are available for this purpose, but any cleanable, leak-proof container with a lid may be used. Waste collection bags are not to be removed from these secondary containers until such time as they are to be placed directly into the autoclave or some other secondary container (shallow tub or tray, without lid) in preparation for autoclaving.
- 7.1.3 Autoclavable bags should not be filled beyond approximately  $\frac{3}{4}$  full, at which point they should be secured for autoclaving. It is recommended that autoclavable zip ties be used to secure tops rather than tying the bags off.

### 7.2 Waste Treatment by Autoclaving

- 7.2.1 Remove secured autoclavable bags of waste from secondary container and place in a shallow pan. (If available, a rack that elevates the bag off the bottom of the pan and above the sides of the pan should be placed inside the pan.)
- 7.2.2 Affix a small piece of autoclave indicator tape to the outside of the bag. Alternatively, heat indicator bags may be used.
- 7.2.3 Place the pan and bag inside the autoclave. Do not overload the autoclave; there should be at least 2 inches of space around each waste bag on all sides to allow access to surfaces by the steam. No other materials should be autoclaved in the same load.

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**7.2.4** Run the autoclave at a chamber temperature of 121°C for 60 minutes\*, using a dry materials cycle.

\* 121°C is the standard temperature for autoclave operation, and generally achieved when chamber pressure is 15-16 PSI. However, this pressure is dependent upon altitude; at higher altitudes the pressure must be increased to achieve 121°C. It is also important to recognize that the parameters of time and temperature have an inverse relationship; operation at higher temperatures will allow the time to be decreased, and operation at lower temperatures will require longer times. The 60 minute time specified is recommended, but must be validated by testing as described in section #7.3, and adjusted accordingly.

**7.2.5** When the autoclave chamber and ambient pressure are the same, the chamber may be opened and the waste bag removed. Wear autoclave gloves when handling hot items. Also use caution when opening the chamber door, as hot steam will be released from the chamber.

**7.2.6** Place the treated waste bag inside a black plastic bag. The treated waste may now be discarded in the normal solid waste stream.

### **7.3 Validating Autoclave Performance**

**7.3.1** Each autoclave must have a functional monitoring or measuring device to ensure that the recommended temperature is achieved for the proper length of time on each load.

**7.3.2** Each waste bag decontaminated by autoclaving should have a heat-sensitive indicator such as a piece of autoclave tape attached to the outside of the bag.

**7.3.3** At least once each month, autoclaves used to decontaminate wastes shall be tested by using endospores from the bacterium *Geobacillus stearothermophilus* in a challenge test. Procedure for challenge test:

**7.3.3.1** Testing should be done using BT Sure™ (Barnstead / Thermolyne, Inc., Debuque, IA) test vials, which can usually be read in 24 hours.

**7.3.3.2** A vial containing a known number of endospores (e.g.,) is buried within the center of a bag of waste. This is facilitated by tying a piece of string to the vial and leaving the other end of the string trailing out the opening of the bag.

**7.3.3.3** The waste bag is secured and autoclaved according to the standard operating procedure detailed in section 7.2 above.

**7.3.3.4** After the bag has cooled, the vial is retrieved and incubated at 55-60°C for the appropriate time required to read the results, up to 48 hours. If the vial contents turn from purple to yellow, this indicates viable spores survived the autoclaving process, grown, and produced acid by fermentation.

**7.3.3.4.1** If after 24 hours: yellow – failed the test (definite); purple – presumptive passed the test; continue to incubate up to 48 hours.

**7.3.3.4.2** If after 48 hours: vial remains purple, passed the test (definite, all spores killed).

**7.3.3.5** Test results, date, and run parameters of the test shall be recorded.

**7.3.4** Environmental Health & Safety manages a campus-wide testing program for waste treatment autoclaves using the procedure described above. Users obtain a test kit (with vial) from EH&S, fill out the data card, bury and retrieve the vial in a bag of waste, then return the vial to EH&S by campus mail. EH&S will incubate the vial, read the results, and generate a report to the user. There is no charge for this program, and autoclave users are encouraged to participate. Contact EH&S to receive a test kit.

### **7.4 Detection Limits**

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- 7.4.1 Testing by simple chemical indicator will tell only if the autoclave has reached the approximate normal operating temperature, but will not tell how long that temperature was maintained.
- 7.4.2 Validating performance by use of recording devices will tell both time and temperature, but will not ensure that materials within the center of the bag have been sterilized.
- 7.4.3 Validation by use of biological indicator spores is the only way to ensure complete sterilization.

**7.5 Infectious Waste Storage Times and Temperatures**

- 7.5.1 Biological wastes and cultures / stocks must be autoclaved within seven (7) days of generation, unless refrigerated at temperatures between 33-48°F or frozen at temperatures below 32°F.
- 7.5.2 Refrigerated or frozen waste must be treated within 30 days of generation.

**7.6 Precautions**

- 7.6.1 Always wear thermal protection gloves when handling items that have recently been autoclaved.
- 7.6.2 Use caution when opening the door of the autoclave after a run, as steam will be released.
- 7.6.3 Personnel must use precautions to ensure placement and retrieval of test vials within a bag of potentially infectious waste does not result in exposures to infectious materials. Precautions should include as a minimum the use of appropriate PPE and mechanical methods (forceps, etc.) to place and retrieve the vial within the bag. If necessary, the vial may be run inside a bag of waste that has been “pre-cooked” by autoclaving.

**8.0 REPORTING AND DOCUMENTATION:**

Records of repairs, service calls, and calibrations of autoclaves should be maintained by the user or department.

Users must maintain records of any validation challenge testing as described in Section 7.3 of this document, or by equivalent methods approved by the OSU Biosafety Officer.

Environmental Health & Safety shall generate a report for each autoclave tested through the OSU campus-wide program, and a copy of that report will be transmitted to the user identified on the challenge test data card. In addition, a summary of all tests performed for each autoclave shall be maintained in database form by EH&S.

**9.0 REVIEWS AND REVISIONS:**

This procedure shall be reviewed for compliance and effectiveness by the Biosafety Officer and the Institutional Biosafety Committee and revised as necessary.

**10.0 ATTACHMENTS and REFERENCE FORMS:**

ATTACHMENT A. Representative Test Report Template

ATTACHMENT B Pressure vs. Temperature for water above 100°C

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## Autoclave Test Report

Biosafety Program  
Environmental Health & Safety

### **Test Vial Information**

Test vial #:

Test vial type: BT Sure / Barnstead Thermolyne

Test vial Lot #:

Expiration Date:

Each vial contains  $2.0 \times 10^5$  endospores, *Geobacillus stearothermophilus*

Incubation conditions: 55°C for >24 hours

### **Autoclave Information**

Building:

Room:

Manufacturer:

Model:

Serial:

### **Test Conditions**

Time:

Chamber Pressure:

Buried in waste bag:

Date of test:

**Results:**

### **User Information**

Department:

Contact Person:

Comments:

Pressure and temperature for water are related by the following equation:

$$\ln P = \frac{-\Delta H_{\text{vap}}}{R} \left[ \frac{1}{T} \right] + C$$

where,             $\ln P$  is the natural log of pressure,  
                       $R$  is the universal gas constant, 8.314 J/mol·K  
                       $\Delta H_{\text{vap}}$  is the enthalpy of vaporization, 40.7 kJ/mol at 100°C  
                       $T$  is absolute temperature, in kelvin  
                       $C$  is a constant, 19.7534 for water

To calculate  $P$  for the chamber, subtract the value for atmospheric pressure for the calculated value obtained from the above equation. Atmospheric pressure = 760 mm Hg (torr), 14.7 psi, 1 atm at sea level.

Temp (C)	Temp (K)	Temp (F)	P (torr)	P (psi)	Gauge P
					P (psi) - Patm
100	283.15	212.0	760.00	14.696	0.000
101	284.15	213.8	787.51	15.228	0.532
102	285.15	215.6	815.86	15.776	1.080
103	286.15	217.4	845.12	16.342	1.646
104	287.15	219.2	875.06	16.921	2.225
105	288.15	221.0	906.07	17.521	2.825
106	289.15	222.8	937.92	18.136	3.440
107	290.15	224.6	970.60	18.768	4.072
108	291.15	226.4	1004.42	19.422	4.726
109	292.15	228.2	1038.92	20.089	5.393
110	293.15	230.0	1074.56	20.779	6.083
111	294.15	231.8	1111.20	21.487	6.791
112	295.15	233.6	1148.74	22.213	7.517
113	296.15	235.4	1187.42	22.961	8.265
114	297.15	237.2	1227.25	23.731	9.035
115	298.15	239.0	1267.98	24.519	9.823
116	299.15	240.8	1309.94	25.330	10.634
117	300.15	242.6	1352.95	26.162	11.466
118	301.15	244.4	1397.18	27.017	12.321
119	302.15	246.2	1442.63	27.896	13.200
120	303.15	248.0	1489.14	28.795	14.099
121	304.15	249.8	1536.80	29.717	15.021
122	305.15	251.6	1586.04	30.669	15.973
123	306.15	253.4	1636.36	31.642	16.946
124	307.15	255.2	1687.81	32.637	17.941
125	308.15	257.0	1740.93	33.664	18.968
126	309.15	258.8	1795.12	34.712	20.016
127	310.15	260.6	1850.83	35.789	21.093
128	311.15	262.4	1907.83	36.891	22.195
129	312.15	264.2	1966.35	38.023	23.327
130	313.15	266.0	2026.16	39.180	24.484
131	314.15	267.8	2087.42	40.364	25.668
132	315.15	269.6	2150.42	41.582	26.886
133	316.15	271.4	2214.64	42.824	28.128
134	317.15	273.2	2280.76	44.103	29.407
135	318.15	275.0	2347.26	45.389	30.693
136	319.15	276.8	2416.34	46.724	32.028
137	320.15	278.6	2488.16	48.113	33.417
138	321.15	280.4	2560.67	49.515	34.819
139	322.15	282.2	2634.84	50.950	36.254
140	323.15	284.0	2710.92	52.421	37.725
141	324.15	285.8	2788.44	53.920	39.224
142	325.15	287.6	2867.48	55.448	40.752
143	326.15	289.4	2948.80	57.020	42.324
144	327.15	291.2	3031.64	58.622	43.926
145	328.15	293.0	3116.76	60.268	45.572
146	329.15	294.8	3203.40	61.944	47.248
147	330.15	296.6	3292.32	63.663	48.967
148	331.15	298.4	3382.76	65.412	50.716
149	332.15	300.2	3476.24	67.220	52.524
150	333.15	302.0	3570.48	69.042	54.346

## Autoclave Pressure vs Temperature

