



Policies and Procedures Reference

STERILIZATION VERFICATION BY UTLIZATION OF BIOLOGICAL INDICATORS

Questions and comments related to this guide can be directed to:

Chemical Control Centre Laboratory Safety Division 401 Sunset Avenue Windsor, Ontario N9B 3P4 e-mail: <u>ccc@uwindsor.ca</u> web: uwindsor.ca/labsafety

Overview:

This guide is designed to outline the policies and procedures associated with the testing of institutional sterilizers to ensure that they are working in accordance with various municipal, provincial, and federal guidelines. The primary goal of this guide is to increase awareness of issues related to the treatment of biological materials in a safe, efficient, and effective manner.

Individual departments on campus may have more stringent polices and procedures depending on the materials used in their research and teaching programs. In addition, specific individuals have been designated with the role of ensuring their departmental or research unit is operated properly, maintained regularly, records management, and that users are trained. Therefore, it is important to meet with the proper individual within your department prior to using the instrument. If you are unable to determine who the appropriate point of contact is within your department, please contact the Chemical Control Centre (ext. 3523).

Contacts:

Department	Telephone Ext.
Chemical Control Centre – Client Services	Ext. 3523

Departmental Contact	Telephone Ext.

Biological Safety Committee (2005 – 2007):

Name	Department	Telephone Ext.
Mrs. Leigh Harold	Occupational Health & Safety	Ext. 2055
Mr. Chris Busch	Chemical Control Centre	Ext. 3524

Responsibility:

Individual research and academic laboratories who utilize potentially hazardous biological agents and materials are responsible for the separation, packaging, and treatment of their own biological waste. It is the responsibility of the individual researcher or department who is in control of the instrument to ensure that the proper verification process have been completed prior to treatment of biological materials.

1. Overview:

The Ministry of the Environment is the provincial authority responsible for the treatment and disposal of biomedical waste under the Environmental Protection Act. Under Ontario Regulation 558, residual wastes which are derived from the treatment of biomedical waste remain biomedical waste unless treated by methods such as incineration, steam sterilization, and chemical disinfection. It is important to ensure that the equipment utilized in the rendering of biomedical waste to its non-hazardous form is capable of properly sterilizing or disinfecting the material. To ensure successful sterilization, the University of Windsor has developed an internal quality control system to validate the ability of the institution's autoclaves to treat biological waste.

Therefore, this guide has been created to outline the necessary processes which are to be completed on campus as it relates to the monitoring of steam sterilization using the AttestTM biological monitoring system. This commercial system has been commonly used in both hospital and dental practices for over twenty years.

2. <u>Definitions:</u>

To ensure accurate communication related to biological materials the University of Windsor has adopted the following definitions when explaining process of decontamination for Biosafety:

Biological Incubator: The Chemical Control Centre provides free-of-charge a 3M Attest biological incubator for the accurate incubation of both the test sample and positive control used in verification of sterilizer efficiency (CCC PART # LAB1256).

Decontamination Any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

Disinfectant A chemical or mixture of chemicals used to kill microorganisms, but not necessarily their spores. Disinfectants are usually applied on inanimate surfaces or objects.

Disinfection A physical or chemical means of killing microorganisms, but not necessarily their spores.

Sterilization A process that destroys and/or removes all classes of microorganisms and their spores.

3. <u>Background Information:</u>

The biological indicators are comprised of self contained devices comprised of five major components, including:

- Dry Spore Strip: contains spores of *Bacillus stearothermophilus* with a minimum population of $1 \ge 10^5$ spores per strip.
- Growth Media: contained within a crushable glass ampoule within the vial. The media is comprised of a Tryptic Soy broth with a pH indicating dye (bromocresol purple).
- Vial: comprised of polypropylene and is used to encapsulate both the dry spore strip and media.
- Brown cap: is comprised of a hydrophobic filter to allow for air to permeate while maintaining sterility within the vial.
- Label: identifies the date of manufacturing and changes colour when sterilization is completed (pink to brown). In addition, space is available to record tracking information.

Biological Indicator Test Package – CCC PART # LAB1244

Chemical Control Centre:

Customer Service: Hours of Operation: E-mail: Webpage:

Ext. 3523 (p) / 519.973.7013 (f) 8:30AM - 4:30PM ccc@uwindsor.ca www.uwindsor.ca/ccc

Order on-line via our website (ROES), by phone, fax, e-mail, or inter-office mail.

4. <u>Procedure – Steam Cycle (Unwrapped):</u>

Unwrapped packs contain items which are to be sterilized at $250 - 254^{\circ}F$ ($121 - 123^{\circ}C$) for 15 minutes or more in a gravity displacement cycle, $270 - 274^{\circ}F$ ($132 - 135^{\circ}C$) for 3 minute or more in a pulsing or pre-vacuum cycle, or $285 - 287^{\circ}F$ ($141 - 143^{\circ}C$) for 2 minutes or more in a pulsing cycle.

- 4.1. Identify the indicator by noting the sterilizer and load numbers, and the processing date on the label.
- 4.2. Place a 1262P Attest[™] biological indicator in an unwrapped basin, tray, or any unwrapped metal item that is representative of the load to be sterilized.
- 4.3. Place the test pack in a full load in the area most challenging for the sterilizer. In a steam sterilizer this is generally on the bottom shelf, near the door, and over the drain.
- 4.4. Initiate the sterilization cycle. Record sterilization cycle within Daily Autoclave Log (see appendix).
- 4.5. After completion of the sterilization cycle and while wearing safety glasses, lab coat, and gloves, completely open the exterior door of the autoclave for five (5) minutes.

It is important that you do not handle the biological indicator until the ampoule has cooled. Excessive handling may result in personal injury caused by flying debris.

Appropriate safety equipment (gloves, eye protection, and lab coat) must be used when removing biological indicators from the sterilizer.

- 4.6. Carefully retrieve the indicator from the test package.
- 4.7. Examine the chemical label located on the Attest Biological Indicator label. If the indicator has changed from pink to brown then the temperature within the sterilizer was sufficient. Chemical indicators do not indicate that the sterilization process was successful.
- 4.8. If incubation can not be completed immediately, refrigerate the biological indicator until incubation can be completed. For incubation instructions, see section 7 Incubation.

5. <u>Procedure – Steam Cycle (Wrapped):</u>

Wrapped packs contain items which are to be sterilized at $250 - 254^{\circ}F(121 - 123^{\circ}C)$ for 20 minutes or more in a gravity displacement cycle, $270 - 274^{\circ}F(132 - 135^{\circ}C)$ for 10 minute or more in a pulsing or pre-vacuum cycle, or $285 - 287^{\circ}F(141 - 143^{\circ}C)$ for 3 minutes or more in a pulsing cycle.

- 5.1. Identify the indicator by noting the sterilizer and load numbers, and the processing date on the label.
- 5.2. Place a 1262P Attest[™] biological indicator in the centre of a representative wrapped hard goods package (i.e. tips/tubes) of the sterilizer load.
- 5.3. Place the test pack in a full load in the area most challenging for the sterilizer. In a steam sterilizer this is generally on the bottom shelf, near the door, and over the drain.
- 5.4. Initiate the sterilization cycle. Record sterilization cycle within Daily Autoclave Log (see appendix).
- 5.5. After completion of the sterilization cycle and while wearing safety glasses, lab coat, and gloves, completely open the exterior door of the autoclave for five (5) minutes.

It is important that you do not handle the biological indicator until the ampoule has cooled. Excessive handling may result in personal injury caused by flying debris.

Appropriate safety equipment (gloves, eye protection, and lab coat) must be used when removing biological indicators from the sterilizer.

- 5.6. Carefully retrieve the indicator from the test package.
- 5.7. Examine the chemical label located on the Attest Biological Indicator label. If the indicator has changed from pink to brown then the temperature within the sterilizer was sufficient. Chemical indicators do not indicate that the sterilization process was successful.
- 5.8. If incubation can not be completed immediately, refrigerate the biological indicator until incubation can be completed. For incubation instructions, see section 7 Incubation.

6. <u>Procedure – Steam Cycle (Fabric Packs):</u>

Fabric packs contain items which are to be sterilized at $250 - 254^{\circ}F(121 - 123^{\circ}C)$ for 30 minutes or more in a gravity displacement cycle, $270 - 274^{\circ}F(132 - 135^{\circ}C)$ for 3 minute or more in a pulsing or pre-vacuum cycle, or $285 - 287^{\circ}F(141 - 143^{\circ}C)$ for 2 minutes or more in a pulsing cycle.

- 6.1. Identify the indicator by noting the sterilizer and load numbers, and the processing date on the label.
- 6.2. Place a 1262P Attest[™] biological indicator between two small packs from the sterilizer load which when taken together approximate the largest and most dense pack within the load. Over wrap the packs in paper and label "Test Pack".
- 6.3. Place the test pack in a full load in the area most challenging for the sterilizer. In a steam sterilizer this is generally on the bottom shelf, near the door, and over the drain.
- 6.4. Initiate the sterilization cycle. Record sterilization cycle within Daily Autoclave Log (see appendix).
- 6.5. After completion of the sterilization cycle and while wearing safety glasses, lab coat, and gloves, completely open the exterior door of the autoclave for five (5) minutes.

It is important that you do not handle the biological indicator until the ampoule has cooled. Excessive handling may result in personal injury caused by flying debris.

Appropriate safety equipment (gloves, eye protection, and lab coat) must be used when removing biological indicators from the sterilizer.

- 6.6. Carefully retrieve the indicator from the test package.
- 6.7. Examine the chemical label located on the Attest Biological Indicator label. If the indicator has changed from pink to brown then the temperature within the sterilizer was sufficient. Chemical indicators do not indicate that the sterilization process was successful.
- 6.8. If incubation can not be completed immediately, refrigerate the biological indicator until incubation can be completed. For incubation instructions, see section 7 Incubation.

7. Incubation:

7.1. If incubation could not be completed immediately, remove the biological indicator from refrigeration and place the bottom of the indicator into the metal block of the Attest incubator at a 45-degree angle (Figure 1).



7.2. Apply slight pressure to the indicator and push straight back (Figure 2). This step crushes the glass ampoule within the vial and "actives" the indicator.



7.3. Push the active indicator into the metal heating block (Figure 3). Verify that the hydrophobic cap (brown) remains above the metal block.

Figure 3	

- 7.4. Repeat the steps above with a non-sterilized biological indicator. This positive control must be from the same lot and manufacturing date as the test indicator. The positive control will ensure that incubator is functioning properly, biological indicator is viable, and the Tryptic Soy broth is capable of promoting growth of the *G. stearothermophilusi*.
- 7.5. After 8-hours, examine both the control and test indicators for a colorimetric change (yellowing). Continue to examine the indicators for bacterial growth has 12, 18, 24 and 48-hours. For interpretation of your results, see the figure below.

Visual Observation	Interpretation
Results Negative Test (purple) Positive Control (yellow)	Sterilization Successful
Positive Test (yellow) Positive Control (yellow)	Sterilization Failed – Do not process any other loads
Negative Test (purple) Negative Control (purple)	Quality Control Failure (Spore Viability) – Repeat Test
Figure 4	

- 7.6. Record your results in the University of Windsor's Biological Indicator Test Record (see Appendix).
- 7.7. Place both positive control and test samples within the General Biomedical Waste stream. **Do not discard biological indicators in general garbage.**

8. <u>Troubleshooting:</u>

There are numerous reasons why a sterilization cycle was not successful, including sterilizer performance, material quality and quantity, choice of packaging materials, packaging technique, sterilizer loading techniques, and inappropriate cycle parameters for the items being processed.

The parameters needed for steam sterilization are time, temperature and saturated steam. A steam sterilization process failure can be caused by poor steam quality and/or inadequate steam quantity, equipment malfunction and human error.

Poor steam quality and/or inadequate steam quantity, caused by:

Wet steam: inadequate trap in steam line, steam contact with a cold load, steam pressure too high for the temperature

Superheated steam: improper chamber heat up, desiccated packaging materials, steam pressure too low for the temperature;

Variations in steam pressure: due to clogged filters, poorly engineered piping or excessive demands.

Out-of-calibration pressure gauges and controllers: Incomplete air removal, plugged drain screen, clogged vent lines, faulty vacuum pump, inadequate door gasket seal, low steam pressure, plugged, faulty or maladjusted control valves, come up time less than 1.5 minutes

Inadequate cycle temperature: temperature gauge out of calibration, long heat-up time of large loads (heat lag), variations in steam pressure due to clogged filters, poorly engineered piping or excessive demands on the steam supply;

Insufficient time at temperature: timer gauge out of calibration, inappropriate cycle parameters for the load being processed, come up time less than 1.5 minutes,

Human error: inadequately cleaned items preventing steam penetration, packaging materials impermeable to steam, packs too large or too dense for the cycle parameters, poor loading techniques that entrap air and prevent steam penetration, incorrect operation of sterilizer entire load inadvertently not processed.

Please refer the University of Windsor's Policies and Procedures for Autoclaves for more information. In addition, please contact either your departmental sterilization contact or the Chemical Control Centre ext. 3523 for more information.

9. <u>References:</u>

Biological Monitoring: Does your system tell you all you need to know? 3M Infection Control Rounds, March, 1987, pp. 1-4.

Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, 4th edition. April 1999.

Guideline C-4. Management of Biomedical Waste in Ontario, Environmental Protection Act, SO 1990 Ministry of the Environment.

Guideline C-17 Non-Incineration Technologies for Treatment of Biomedical Waste (Procedures for Microbiological Testing), Environmental Protection Act, SO 1990 Ministry of the Environment.

Laboratory Biosafety Guidelines, Public Health Agency of Canada, 3th Edition, 2004.

Laboratory Biosafety Manual, 2nd edition (revised), Global Health Security – Epidemic Alert and Response, World Health Agency. Geneva, 2003.

10. <u>Contact Information:</u>

For more information, please contact:

Chemical Control Centre Laboratory Safety Division Essex Hall, B-37 p. 519.253.3000.3523 e. <u>ccc@uwindsor.ca</u> w. www.uwindsor.ca/labsafety **Daily Autoclave Log**

Model: _

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01	

Room: _

Building: _

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Length of exposure (min.)										
Temperature Achieved (°C)										
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Autoclave Operator										ve records for audit
Description of Contents										lease place the completed training document with your autock
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Name of Generator (Principle Investigator)										Per
Date										

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Autoclave Performance Records	Building:	Description of Service Call								
	Room:	Comments								our autoclave records for audit purposes.
	: Serial Number:	Remedial Action								Please place the completed training document with y
	Make: Model	Problem								Person-in-charge: 1
	Autoclave:	Date								