Safety Resources

Procedure Title: Biological Indicators – Monitoring the Effectiveness of Autoclaves

1. Introduction

It is mandatory to validate the effectiveness of waste sterilization monthly for every autoclave using biological indicators. Biological indicators (BIs) must be used to ensure an autoclave load reaches the correct temperature for the correct amount of time. This will ensure sterilization of the biohazardous waste. The purpose of this procedure is to describe the monitoring of efficacy testing and the quality control of an autoclave with the use of biological indicators.

2. Definition

BIs	Self-contained biological indicator vials (BIs) containing Geobacillus stearothermophilus, or and *B. atrophaeus*, and medium with a pH indicator to detect growth. If the autoclave does not reach the right temperature, the spores survive the sterilization cycle and when incubated in the growth media, the spores will germinate and their metabolic byproducts will change the colour of the pH sensitive media. These indicators are used to develop the processing times for typical loads and monitor the efficiency of the decontamination processes. BI can also be used to confirm thermocouple data when checking heating profiles and validating autoclave efficacy.
PPE	Personnel Protective Equipment
SOP	Standard Operating Procedures

3. Safety

3.1. Manuals for the autoclave and biological indicators should be readily available in close proximity to the autoclave.

3.2. Autoclave User logs and Autoclave User Log should be filled out after each use (Refer to Appendix.)

3.3. Since there is a glass ampule in the biological indicator package, follow the safety procedures.

- Handle hot BIs with care - Crushing or excessive handling before cooling may cause glass ampule to burst.
- Wear safety glasses and disposable gloves when removing the BI from the sterilizer.
- Handle BI by cap only.
- Do not use your fingers to crush glass ampule. Use the incubator to break the ampule.
- Wear safety glasses and gloves when crushing the BI when placing into the BI incubator.
- Do not roll BI between fingers to wet spore strip.

4. Procedure

4.1. Monitoring of autoclaves with BIs should be done monthly.

4.2. Write the date and the load number on the BI.

4.3. Place the BI in the centre of the load. **(NOTE: Each load must be tested individually when developing processing times and temperatures)**

4.3.1. Liquid Loads – Attached BI to a wire and place into the bottom of the vessel containing the test liquid (the liquid at the bottom of the vessel takes the longest to reach sterilization temperature).

4.3.2. Dry or Semi-dry Loads – Attach BI to a long string or wire and place the BI into the the autoclave bag with the string hanging out of the bag; when the cycle is done, retrieve the BI by pulling on the string.

4.4. Put a piece of autoclave tape on the surface of the autoclave load.

4.5. Place the temperature probe into the load (if applicable), the BI package, and the load in to the autoclave according to autoclave SOP in place.

4.6. Run the cycle to sterilize the load as described in the autoclave SOP or manufacturer's instructions.

4.7. When the cycle is complete, open the autoclave as per protocol and remove the BI. NOTE: Wear appropriate PPE – wear lab coat, autoclave gloves, etc.).

4.8. Allow BI to cool for a minimum of 10 minutes.

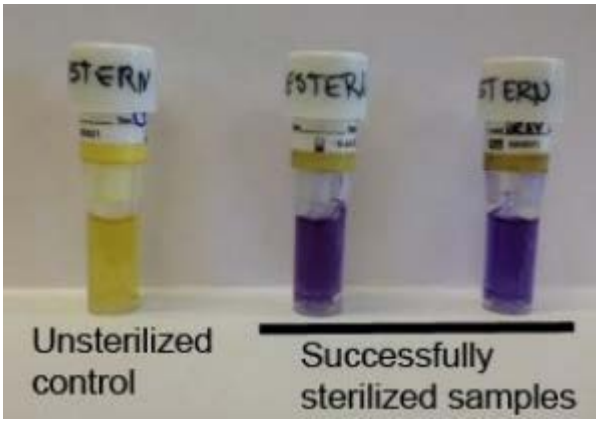
4.9. Place the autoclaved waste in a holding area for the duration of the incubation time of the BI until the results of the cycle have been determined.

4.10. Check the chemical indicator strip on the BI for a change in color, if applicable. (Some BI brands include a chemical indicator on the label that will change from one color to another (e.g. Rose to brown) upon exposure to the steam during the sterilization process). If the chemical indicator strip has not changed colour, repeat the sterilization cycle with a new BI.

4.11. Activate (crush) autoclaved BI by inserting into incubator at 45° angle, as per manufacturer's instructions. Ensure the spore strip is wetted entirely with the growth medium.

4.12. Incubate autoclaved BI along with a non-autoclaved BI with the same lot number as a positive control at the appropriate temperature. Refer to manufacturers' instructions for specific incubation time and temperature. Once incubation is completed, compare the autoclaved BI to the positive control (refer to examples of comparison below).

Comparison of Process BI to Positive Control BI:



Examples of Commercially Available Tests:

4.12.1. **Getinge Biosign BIs:** Incubate BI at 55°C for 24-48 hours. Check the BI after 48 hours, if NO colour change occurs, the sterilization cycle has PASSED; if the colour change in the media from red to yellow, sterilization cycle has FAILED. (E.g. Parameters of time and/or temperature have not been met in the test indicator)

4.12.2. **Raven ProSpore BIs:** Incubate BI at 55°C for 24 or 48 hours (dependent on the kit). During this time, if NO colour change occurs, the sterilization cycle has PASSED; if the colour change in the media from purple to yellow, sterilization cycle has FAILED.

4.12.3. **3M ATTEST Biological System (for steam autoclaves) BIs (refer to appendix 2):** Incubate BI at 55°C for 24 hours (1261P) or 48 hours (1261). During this time, if NO colour change

occurs, the sterilization cycle has PASSED; if the colour change in the media from purple to yellow, sterilization cycle has FAILED.

4.12.4. For the **3M ATTEST Rapid Readout BIs:** Incubate BI at 60°C for 3 hours; Fluorescence detected by 3M ATTEST Auto-reader indicates growth and sterilization failure.

Once the results have been determined and sterilization process was successful, throw the autoclaved waste in regular garbage. (NOTE: For janitors to pick up the garbage in an autoclave bag, it must be covered with a regular garbage bags such that the biosafety label cannot be read or have the Biohazard label DEFACED PRIOR to throwing out in regular garbage). If the sterilization process FAILED (eg. growth seen in the BI), then the autoclave load needs to be RE-autoclaved; repeat the procedure with a new BI. For any of the above BI based tests, the USED BI from FAILED tests should also be re-autoclaved and then disposed.

4.13. Discard incubated BIs into SHARPS container and autoclave prior to disposal.

4.14. Record the BI results in the Autoclave User Log (see Appendix 1).

4.15. Failure to achieve sterilization may be due to:

4.15.1. Improper loading or overloading of the autoclave so that the centre of the load failed to achieve sterilization temperatures.

4.15.2. Insufficient sterilization time to attain 4-6 $\log_{10}$ reduction of the bacterial spores of the BI.

4.16. In the event of FAILED sterilization, repeat the process until necessary loading configuration and sterilization times have been achieved.

4.17. Effective time and load configuration must be used for all subsequent cycles for that type of load.

4.18. Monitoring of the autoclave with BIs must be done biweekly or dependent on the frequency of use (e.g. If autoclave used twice per month then should monitor the efficacy of the autoclave each time a load is done).

5. Equipment or Materials Required

5.1. BIs (e.g. 3M ATTEST, Raven Prospore, or Getinge Biosign)
5.2. Incubator (e.g. 3M ATTEST, Raven Prospore, or Getinge Biosign)
5.3. Autoclave User Log
5.4. Autoclave tape
5.5. Autoclave thermometer probe (if applicable)
5.6. Autoclave Biohazard bags
5.7. SHARPS Container

6. Highlights / Critical Control Points

6.1. Labels and tape indicators for steam, time, and temperature are useful for day to day monitoring BUT are not indicators for sterilization.

6.2. Temperature (thermocouple) probes, placed at the centre of the load may be used to monitor the internal temperature of that load; these may be used in conjunction with a BI.

7. Reporting

The operator of the autoclave MUST maintain the Autoclave User Log.

8. Regulatory / Standards

8.1. BIs MUST be included in autoclave load monthly, or more frequent (dependent on the frequency of use).

A non-autoclaved BI must be incubated as a positive control along with the autoclaved indicator. Ensure same lot number is used for the test and positive control BIs. Record the lot number and results on the Autoclave user log.

8.2. Load testing

8.2.1. Routine monitoring is to be conducted to ensure proper settings are attained. Any changes in standard loads must be first tested and approved by the Autoclave operator. Contact the Biosafety Manager if require assistance.

9. Trouble Shooting

Refer to manufacturing instruction for the BI for troubleshooting

10. References

10.1. Autoclave Manufacturer's Instructions
10.2. BIs Manufacturer's Instructions
10.3. Canadian Biosafety Handbook (2nd. Ed., 2016)

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11. Appendix:

Autoclave User Log

Manufacturer:_____ Model: _____
Year: _____

Serial No.:_____ Location:_____

Asset No.:_____ Put in service date: _____

Name	Date/ Time	Program Type (gravity or liquid)	Cycle Time	Cycle Temperature (°C)	Type of Load (e.g. pertri dishes)	Type of BI used (E.g. 3MTest)	BI Expiry Date & Lot #	Results (Pass/Fail)

Appendix 2:

General instructions for BIs (e.g. 3M ATTEST)

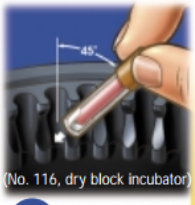
Instructions for Use: Weekly biological monitoring is recommended.[1]

1. Write the sterilizer number, load and date on the indicator.

2. Place (test) indicator into a package that is similar to the typical packs used (e.g., in an autoclave bag) and in the area of the autoclave that is the most difficult to sterilize, i.e., over the drain or in the center of a full load. **Run cycle.**

3. Caution: After sterilization, the contents of Attest biological indicators are hot and under pressure. Always allow to cool. **Failure to cool for at least 10 minutes may cause the glass ampule to burst which may result in injury from flying debris.**

4. Check the chemical indicator on the Attest indicator for a color change from rose to brown.



5. Activate (crush) processed indicator by inserting into the incubator.

6. Use of a Control Incubate a non-processed, activated Attest indicator at the same time. A control:

- ensures spore viability
- demonstrates the capability of the media to promote growth
- confirms that the incubator is functioning properly

7. Examine both the test and the control indicators for any color change at regular intervals and, finally, at 24 hours for the 1261P, or 48 hours for the 1262P indicators.

Act on a positive test (yellow) immediately.

Attest™

Biological Monitoring System — Use at Least Once A Week Per Sterilizer

Date	Sterilizer No. & Type	Attest Cap Color	Date & Time In Incubator/ Initials	Date & Time Out Incubator/ Initials	Results (Circle One)	Control (Circle One)
					+	+
					-	-

8. Record results in the log book.

Indicator Selection for Steam Autoclaves			
Sterilization Process	**Time (minutes)**	**Packaging Materials**	**Attest Indicator (cap color)**
250ºF (121ºC) (steam)	≤15 20 ≥30	None Wrapped Containing fabrics	1262P (Brown) Biological Indicators (25)
270ºF (132ºC) (steam)	≥3 10	None Wrapped	1261P (Blue)* Biological Indicators (25)

*The 1262P (Brown) may be substituted for the 1261P (Blue) when monitoring the 10-minute, 270ºF cycle.

3M™ Attest™
Biological Monitoring System (Item No. 116K)
Includes: one incubator, one 1262P box of 25 indicators and one log book.

Results
Negative Test (purple)
Positive Control (yellow)

Interpretations
Spores were killed. The sterilization process was successful.

Positive Test (yellow)
Positive Control (yellow)

Sterilization process failure. Recall all loads since last negative test. Determine cause for sterilization process failure. Reprocess load. Do not process any other loads until biological indicators test negative in three successive cycles.

Negative Test (purple)
Negative Control (purple)

There is a problem with spore vitality, the growth media or the incubator temperature. Check dating of the Attest indicators used. Repeat test. If results are the same, send incubator in for servicing.

Reference: 1. Miller, C. *JADA*, March 1992.

3M Reliability

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