CDC Division of Laboratory Systems

EXCELLENT LABORATORIES, OUTSTANDING HEALTH.

General Considerations for Biological Indicators in Autoclave Use

January 2026

Introduction

This document provides general considerations and best practices for using biological indicators (BIs) to monitor autoclave performance in sterilizing biohazardous wastes. Adhering to these considerations helps laboratories maintain high decontamination standards and reduce risks of pathogen transmission. Laboratories may also use this document to develop site-specific biological indicator monitoring protocols.

Scope

This document outlines safety precautions, general information on regulated medical waste, the use of biological indicators while autoclaving, and templates for documenting the procedures. It does not cover autoclave validation and the use of chemical indicators.

Disclaimer: This document does not guarantee compliance with any specific regulation at the federal, state, local, or institutional level. The use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Centers for Disease Control and Prevention or the U.S. Department of Health and Human Services.

For questions, please reach out to DLSBiosafety@cdc.gov.

Revision History:

- January 2026; Version 1; Initial posting.

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Roles and Responsibilities

Personnel Role	Responsibility
Biosafety Officer (or Process Leader/Technician)	• Coordinates with laboratory personnel or the designated point of contact (POC) to ensure timely completion of BI testing. • Completes reports and uploads them to the designated storage location after each test. • Troubleshoots failed test results with laboratory personnel.
Laboratory Personnel	• Review reports created by the Biosafety Officer as scheduled (e.g., monthly, quarterly, or annually). • Prepare, distribute, collect, and analyze BI according to the procedure.

Safety Precautions

- Follow standard laboratory safety protocols when preparing, distributing, and processing biological indicators.
- Always wear appropriate personal protective equipment (PPE) when using autoclaves or handling biological indicators (Appendix 1).
- Only trained personnel or designated POCs should place or remove biological indicators from the autoclaves post-cycle.

General Information

Federal and state regulations govern the handling of Regulated Medical Waste (RMW). Terminologies may vary by jurisdiction and may include terms such as biohazardous waste or infectious medical waste. These terms typically refer to wastes contaminated by body fluids or infectious materials that are capable of transmitting diseases. Table 1 summarizes a list of common materials included in RMW; however, this list is not exhaustive. Always follow local regulations and facility protocols to properly dispose of or treat RMW.

Each laboratory is responsible for defining and documenting its waste streams (e.g., solids, liquids, mixed wastes [containing biological, chemical, or radiological hazards], animal bedding, and sharps). The laboratory should also develop contingency plans, implement on-site treatment methods, provide training, maintain records and waste tracking systems, and comply with reporting requirements.

Table 1. Common regulated medical waste components.

Solids	Liquids	Animal Bedding	Sharps
• Pipette tips • Disposable labware • Absorbent pads • Disposable PPE • Petri dishes and agar plates • Biological indicators (used for sterilization validation) • Hazardous waste, such as pathological material • Drugs • Non-hazardous laboratory waste materials, such as paper towels or wipes contaminated with non-infectious substances *Note: Specific items suitable for autoclaving may vary depending on local regulations and facility protocols.*	• Culture media • Contaminated liquid medications • Laboratory reagents and solutions • Body fluids *Note: Not all liquid waste can be autoclaved.*	• Absorbent pads • Shredded paper bedding • Cellulose bedding • Aspen wood shavings • Hemp bedding *Note: Animal bedding suitability for autoclaving depends on material composition, moisture content, and risk of degradation or combustion.*	• Syringe (both with and without needles) • Needles (hypodermic, suture, or acupuncture needles) • Scalpels • Contaminated broken glassware (e.g., glass culture tubes, glass slides) *Note: Sharps should be autoclaved to reduce the risk of injury or infection transmission.*

Biological Indicators

Biological indicators (BIs) are the most reliable tool for verifying autoclave sterilization effectiveness. BIs contain highly resistant spores (e.g., *Geobacillus stearothermophilus)* that are used to assess sterilization performance and confirm that the conditions during a sterilization cycle are achieved.

When to Perform BI Tests

BI tests should be performed:

- Upon receipt of a new batch of BIs from the manufacturer (follow your laboratory quality control procedures).
- At least monthly, or more frequently if required by a risk assessment (Appendix 2).
- After autoclave maintenance or repair (Appendix 4).

BI Testing

- Place the BI in the autoclave and run a full sterilization cycle.
- Post-cycle, incubate the BI according to the manufacturer's instructions.
- Observe color change after incubation:
    - Negative Result (No Color Change): No microbial growth; sterilization was successful.
    - Positive Result (Color Change): Microbial growth detected; sterilization failed.

If the BI Test is Positive (Sterilization Failed)

- Do not collect or dispose of waste from the failed cycle.
- Repeat the BI test (Appendix 3).
- If the retest is also positive, notify the biosafety officer, and suspend autoclave use until maintenance is completed and a successful (negative) BI test confirms sterilization (Appendix 4).

Post-Maintenance Requirements

- Autoclaves must pass a BI test after any repair or maintenance before returning to service.
- Waste pickup can only resume after a negative BI result is confirmed.

Documentation

- Record all BI test results and actions taken in accordance with your laboratory institutional policies.
- Refer to Appendices 2-4 for examples of forms for documentation.
- Whenever there is a failed BI test, document according to your laboratory non-conformity event policy.

Conclusion

Biological indicator testing is essential for verifying that laboratory waste has been effectively decontaminated before disposal. Laboratories should ensure that BIs are always available and used within their expiration date. Ensure that BIs are stored according to the manufacturer's specifications. Regular BI testing ensures proper autoclave function, compliance with waste management regulations, and protects public health.

Resources

- EPA Model Guidelines for State Medical Waste Management Model Guidelines for State Medical Waste Management | US EPA
- Title 49 Code of Federal Regulations 173.197 eCFR: 49 CFR 173.197 -- Regulated medical waste
- Biosafety in Microbiological and Biomedical Laboratories 6th Edition (CDC) Biosafety in Microbiological and Biomedical Laboratories—6th Edition

Appendices

Appendix 1: Recommended PPE Disposition List.

PPE	Process	Disposition	
Transportation gloves (moving cages)	• Cage transport only • Remove upon entering the autoclave room	• Reusable • Remain with the technician • Laundered monthly or as needed	
Heat-Resistant Gloves (unloading)	• Handling decontaminated waste from the autoclave	• Reusable • Remain in the autoclave room • Laundered monthly or as needed	
Latex Gloves (loading or unloading)	• Placing/removing waste in/from the autoclave	• Disposable • Discard after each use as autoclave waste	
Nitrile Gloves (loading or unloading)	• Placing/removing waste in/from the autoclave	• Disposable • Discard after each use as autoclave waste	
Laboratory Coat (or uniform)	• Wear while loading/unloading the autoclave	• Reusable • Replace as necessary	
Safety Glasses	• Wear while loading/unloading the autoclave	• Reusable • Remain with the Technician	
Face Shield (optional except if handling liquids)	• Wear while loading/unloading the autoclave	• Reusable • Remain in the autoclave room	

Appendix 2: Biological Indicator Monthly Testing Report

Year: _____

Purpose:
To document and track monthly testing biological indicator test results as a means of verifying the autoclave's proper functioning and providing a comprehensive overview of its performance over the course of a year.

Building: _____

Room#	Autoclave ID#	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec

Reviewer's Signature: _____

Department Management/Quality Review Signature: _____

Appendix 3: Biological Indicator Retest Report

Date: _____

Purpose:
To document and track the results of testing or retesting biological indicators that have initially failed the sterilization process.

Positive Control

Lot#	Expiration Date	Incubation Start Date/Time	Incubation End Date/Time	Result	Point of Contact Notified (If failed)	Comments

Reviewer's Signature:_____

Department Management/Quality Review Signature: _____

Appendix 4: Autoclave Status Report

Date: _____

Purpose:
To provide a summary of autoclave status, specifically documenting instances when autoclaves failed biological indicators testing, were taken out of service, or put back in service, and when repairs or maintenance were performed.

Building	Room #	Autoclave ID#	Status

Reviewer's Signature:_____

Department Management/Quality Review Signature:_____