



CM SOP #111 – Processing Biological Indicators

Original release date: 05/25/2017 Version: 4 Date last revised: 03/29/2026

I. Purpose & Scope

The purpose of this Standard Operating Procedure (SOP) is to establish standardized procedures for processing biological indicators (BIs) used to verify the effectiveness of steam sterilization (autoclaving) within Comparative Medicine (CM) facilities.

Biological indicators are used as part of the Environmental Monitoring and Quality Assurance Program to confirm that autoclave cycles achieve adequate sterilization conditions. Proper processing and documentation of biological indicators supports:

- Verification of autoclave effectiveness
- Biosecurity and biosafety compliance
- Reliable decontamination of biohazardous and quarantine materials
- Regulatory and accreditation readiness
- Continuous quality assurance of sterilization processes

This SOP applies to all personnel responsible for operating autoclaves and processing biological indicators in CM-managed facilities.

II. Roles & Responsibilities

Comparative Medicine Staff

- Performing biological indicator testing according to this SOP
- Processing biological indicators safely and accurately
- Recording results on required log sheets
- Reporting failed biological indicator tests immediately
- Performing only tasks for which training and competency have been documented

Research Staff, Students, Visitors

- Coordinating with Comparative Medicine when autoclaving biohazardous or quarantine materials
- Following institutional procedures for autoclave use
- Notifying Comparative Medicine staff of unusual sterilization needs
- *Note: Research personnel do not independently process biological indicators unless specifically trained and authorized.*

CM Management

- Ensuring procedures are followed as outlined in this SOP
- Ensuring personnel receive appropriate training and competency verification
- Addressing equipment problems or procedural non-compliance
- Maintaining availability of supplies and functioning equipment

CM Director and AV

- Providing oversight of the sterilization quality assurance program



- Ensuring resources and equipment are available to support biological indicator testing
- Reviewing trends in sterilization performance
- Ensuring compliance with institutional biosafety standards

III. General Notes & Definitions

- Steam sterilization using an autoclave is one of the most effective methods for decontaminating biohazardous and quarantine materials.
- Biological indicators are considered the most reliable method for verifying sterilization performance because they contain highly resistant bacterial spores.
- Biological indicators used in CM facilities typically contain spores of *Geobacillus stearothermophilus* and in some products, *Bacillus subtilis*.
- These organisms are selected because they are more resistant to heat than most environmental microorganisms.
- Biological indicators must be incubated according to manufacturer specifications, typically 56–60°C.
- Biological indicator testing must be performed:
 - With each load of biohazardous or quarantine material
 - At least monthly when autoclaves are used routinely
- Biological indicators should be incubated as soon as possible after cooling.
- For optimal performance the biological indicator incubator must remain plugged in and powered at all times.
- **Biological Indicator (BI):** A device containing heat-resistant bacterial spores used to verify sterilization effectiveness.
- **Control Indicator:** A biological indicator that is not autoclaved and is used to confirm proper incubation conditions.
- **Failed BI:** A biological indicator showing bacterial growth after incubation, indicating possible sterilization failure.

IV. Materials & Equipment

- Biological indicator test vials (e.g., 3M Attest) & incubator meeting manufacturer specifications
- Permanent marker
- CM Form 045: Autoclave log sheet
- CM Form 008: Health and Environment Check Sheet
- Personal protective equipment, including:
 - Lab coat
 - Eye protection
 - Autoclave gloves

V. Procedure

A. Preparation of Biological Indicator

1. Obtain a biological indicator vial appropriate for the autoclave cycle being tested.
2. Using a permanent marker, label the biological indicator with:
 - a) Date



- b) Autoclave location
3. If multiple autoclaves are being tested, ensure each biological indicator is clearly identified by autoclave unit.
4. Verify that the biological indicator incubator is:
 - a) Plugged in
 - b) Powered on
 - c) Set to the correct temperature range

B. Placement of Biological Indicator in Autoclave

1. Place the labeled biological indicator into the autoclave load according to manufacturer instructions. The biological indicator should be placed:
 - a) In the most difficult location for steam penetration
 - b) Typically near the center of the load
2. Run the autoclave cycle according to CM SOPs:
 - a) *104: Autoclaving Procedures*
 - b) *110: Autoclave Effectiveness*

C. Removal and Inspection of Biological Indicator

1. Allow the biological indicator to cool once the autoclave cycle is complete.
2. Remove the biological indicator from the autoclave using appropriate protective equipment.
3. Inspect the biological indicator label color.
 - a) A color change from rose to brown indicates exposure to sterilization conditions (pass).
 - b) An incomplete color change may indicate inadequate sterilization (fail).

D. Activation and Incubation of Biological Indicator

1. Place the biological indicator vial into the incubator heating block.
2. Position the vial at approximately a 45-degree angle, as shown below.
3. Push the vial firmly into the heating block to activate the internal ampule.
4. Confirm the internal ampule has been crushed.
5. Immediately place the biological indicator into the incubator.



E. Preparation of Control Indicator

1. Obtain a new biological indicator vial.
2. Label the vial with "C" to designate control.
3. Activate the control vial using the same method described above.
4. Place the control vial into the incubator.
5. The control vial must not be autoclaved.

F. Incubation and Result Evaluation

1. Incubate biological indicators according to manufacturer instructions, but for a minimum incubation time of 48 hours



2. Inspect the color of both vials.
3. Interpret results as follows:
 - a) Yellow color indicates bacterial growth.
 - b) Purple or original color indicates no growth.
4. Expected results:
 - a) Control vial: yellow
 - b) Autoclaved vial: no color change (pass)



G. Failed Biological Indicator Response

If a biological indicator shows bacterial growth:

1. Immediately notify the FM
2. Record the failed results on *CM Form 008: Health and Environment Check Sheet*
3. Reprocess the affected autoclave load.
4. Remove the autoclave from service if repeated failures occur.
5. Initiate troubleshooting and equipment evaluation.

H. Broken Biological Indicator Response

If the outer tube of a biological indicator breaks:

1. Stop work immediately.
2. Contain the spill.
3. Mark the area to prevent access.
4. Notify personnel working nearby.
5. Follow Environmental Health and Safety cleanup procedures.

I. Record Keeping

- The following records must be maintained:
 - Biological indicator test results
 - Autoclave log sheets
 - Health and Environment Check Sheet (CM#008)
 - Autoclave maintenance records
 - Failed biological indicator documentation
- Record retention requirements:
 - Retain autoclave log sheets for one full year.
 - Archive logs older than one year.
 - Records older than three years may be destroyed.
- Records must be available for inspection by:
 - IACUC
 - Environmental Health and Safety
 - Regulatory or accreditation agencies
- Log records should be reviewed:



- Monthly by Veterinary Technician
- Periodically by Facility Manager
- At least semi-annually during program review

VI. Health & Safety

- Personnel processing biological indicators must:
- Wear appropriate PPE. At minimum:
 - Lab coat
 - Autoclave gloves
 - Eye protection
- Use light pressure when crushing the inner glass ampule.
- Excessive pressure may break the outer tube.
- If a biological indicator breaks:
- Follow spill containment procedures immediately.
- Personnel working with autoclaves must:
 - Avoid direct contact with hot surfaces
 - Use thermal protective equipment
 - Follow established autoclave safety procedures
- In the event of injury:
 - Notify supervisor immediately
 - Complete injury report
 - Seek medical evaluation as needed

VII. References & Attachments

- Biosafety in Microbiological and Biomedical Laboratories, 5th Edition
- CM SOP 104: Autoclaving Procedures
- CM SOP 110: Autoclave Effectiveness
- 3M Attest Manufacturer Instructions
- CM Form 045: Autoclave Log Sheet
- CM Form 008: Health and Environment Check Sheet
- Biological Indicator Processing Flowchart

VIII. Revision History

Revision Date	Revision Number	Summary of Changes
05/07/2021	2	Cleaned up processes
10/17/2023	3	
03/29/2026	4	Updated format, made ADA compliant, streamlined language