



OFFICE OF LABORATORY ANIMAL CARE

Working Instructions

WIN Number:	615	Autoclave Validation Testing	Revision #:	0
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PROCEDURE

All work instructions must be demonstrated by the trainer and performed by the trainee.

1. Autoclave Maintenance:

- a. Perform autoclave maintenance as recommended by the manufacturer. Utilize contract service providers for all autoclaves unless not contracted.

2. Autoclave Validation:

- a. Perform biological indicator testing to validate the effectiveness of an autoclave in the following circumstances and during periodic maintenance as above:
 - When using a new type of packaging material or tray
 - After training new personnel to use the autoclave
 - After repair of an autoclave
 - After any change in the autoclave loading procedure
 - New machine
 - Uncertain that load has reached proper temperature
- b. At every cycle:
 - Use black stripe autoclave tape or steam sterilization strips to verify appropriate temperature, time and pressure have been reached.
 - Record autoclave controls confirming appropriate time and temperature have been reached.
- c. Biological Indicator Testing:
 - Perform biological indicator testing at intervals as indicated above.

- *Geobacillus stearothermophilus* is the indicator organism of choice for steam sterilization.
- Choose the appropriate biological indicator test for the autoclave and cycle being used. i.e. Attest 1262/1262P

d. Dry items

- Place biological indicators for surgical instruments within a cotton wrapped surgical pack or a towel pack/gown.
- Place biological indicators for bedding in the center of a bag of bedding and in a typical load.
- ABSL-2/3 areas, indicators may be placed in a small clear autoclave bag closed in a similar fashion as the bedding bag to mimic the process without contaminating/wasting the autoclaved bedding.
- Always place biological indicators in the area within the load that produces the greatest challenge to air removal and steam penetration. **Note:** In a steam sterilizer this is generally on the bottom shelf, near the door, and over the drain.

e. Liquid items

- Use a biological indicator specifically for liquid cycles.
- Place the biological indicator vial into one of the liquid items and place in the center of the autoclave
- Run the cycle
- Carefully remove the biological indicator once cycle has completed

3. Removal of biological indicators

a. After the autoclave cycle has completed

- Fully open the autoclave door and wait for at least 5 minutes before removing the biological indicator.
- Wear safety goggles and heat resistant gloves when removing the biological indicator.
- If the indicator is inside a surgical pack, break sterility by opening the surgical packs when removing indicators.
- Check the chemical indicator on the biological indicator for a color change (e.g., rose to brown).

b. Complete the Autoclave Cycle form and place it in a box next to the incubator along with the biological indicator vials.

4. Incubation of biological indicators

- Incubate the biological indicator at least two (2) hours within completion of the sterilization cycle.
- Activate biological indicators per manufacturer's instructions.
- Place in a metal block in an upright position with the cap remaining above the metal block.
- Incubate the biological indicator per manufacturer's instructions

- c. Incubate a biological indicator control as a positive growth control. **Note:** The control must be of the same lot and manufacturing date as the test biological indicators used.
- d. Incubate at appropriate times per manufacturer's recommendations (e.g., Attest 1261P: 24 hours and Attest 1262P: 48 hours).
- e. Examine the incubators at 24 and 48 hours and interpret results:
 - Successful sterilization:
 - The control will turn yellow, and the sterilized biological indicator test remains purple.
 - Faulty biological indicator:
 - The control remains purple, this indicates that biological indicators were faulty and biological indicator testing must be repeated.
 - Failed sterilization:
 - The control and sterilized biological indicator turn yellow. Contact your supervisor for further directions.
 - All previous items sterilized since the last good biological test must be considered unsterile.
- g. Record results in log provided

5. Bowie Dick Test:

- a. Place a small disposable Bowie Dick test pack into the autoclave (empty chamber) on the lowest shelf above the drain and run the autoclave at a 4-minute sterilization cycle.
- b. Pass test:
 - Complete color change to black.
- c. Failed test:
 - Lack of or a partial color change.
 - Contact your supervisor for further directions.
- d. Record results in the log

6. Autoclave tape:

- a. Utilize autoclave tape in each steam sterilization load.
- b. Place autoclave tape outside of a load in an area that produces the greatest challenge to air removal and steam penetration.
- c. Sterilization cycle completed
 - Confirm the autoclave tape has turned black to indicate sterilization.
- d. If tape does not change to the appropriate color, this indicates that sterilization did not occur. **Note:** the color varies based on the brand of indicator.
- e. Contact your supervisor for further instructions.
- f. Autoclave tape visually indicates a temperature of 121°C for ten (10) minutes or two (2) minutes at 132°C have been reached in a steam sterilizer.

7. Steam Sterilization Indicator Strips:

- a. Surgical and veterinary supplies:

- Place one steam sterilization indicator strip in each cloth pack.
 - Paper pouch
 - Place one steam sterilizer indicator strip in at least one pouch per load.

Note: The steam sterilization indicator strip should be easily visualized without opening the pouch.
 - Place one steam sterilization indicator strip under the load in the autoclave.
 - ABSL-3 materials: Tape steam sterilization indicator strip to outside of load.
- b. Nesting and bedding materials:
- Place a steam sterilization indicator strip in the middle of the load if possible.
- Note:** If it is not possible to place a steam sterilization indicator strip within the load, place it on the outside of the load.
- c. After the sterilization cycle has completed, confirm that the visible steam sterilization indicator strips have changed to the appropriate color. **Note:** The color change will vary with brand.
- d. If the sterilization strip does not change to the appropriate color, this indicates that adequate sterilization did not occur. Contact your supervisor for further instructions.
- e. ABSL-3, nesting, and bedding materials:
- f. Place steam sterilization strip(s) in an autoclave log or calendar and label with date.

REFERENCE DOCUMENTS

REVISION HISTORY			
REVISION NUMBER	AUTHOR(S)	EFFECTIVE DATE	REVISION(S)