Autoclave Operation for Non-Biohazardous Materials	SOP #: RSP.011.01
Effective Date	12/13/2019
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1. Purpose

The purpose of this document is to provide a standard operating procedure for use of the LabStar 70 Basic Lab autoclave at the Burrell College of Osteopathic Medicine (Burrell) BioScience Research Laboratory (BSRL). The LabStar 70 Basic Lab autoclave in the BSRL is approved for the sterilization of equipment, glassware, plasticware, liquids, and media. The LabStar 70 Basic Lab autoclave is not approved for decontamination of potentially infectious waste or biohazardous waste. This standard operating procedure (SOP) outlines procedures related to safe autoclave operation, training of autoclave users, verification of autoclave operation, validation of autoclave performance, and protocols for managing an autoclave that is not operating correctly.

2. Related Policy/Authority

3. Faculty/Staff Responsibilities

3.1 Users: All users are responsible for operating the autoclave in accordance with the parameters outlined in this SOP and immediately report any problems to the Director of Laboratories or Scientific Research Associate.

3.2 Scientific Research Associate (SRA): Responsible for ensuring autoclave is properly maintained and that users are adequately trained on this procedure.

4. Definitions/Abbreviations

4.1 Autoclave – is a steel chamber apparatus designed for sterilizing with steam and high pressure.

4.2 Biological indicator (BI) - uses spores of a thermophilic bacterium to demonstrate a microbiological kill at the end of the autoclave decontamination cycle. A biological indicator provides information on whether necessary conditions were met during the autoclaving process to kill a specified number of microorganisms thereby providing a high level of confidence in the sterilization process.

4.3 Biohazardous Waste - is any liquid or solid waste generated through the handling of specimens from humans or animals that may contain infectious agents. Cultures of infectious agents, human anatomical remains, and animal carcasses that may be infectious are also considered biohazardous waste.

4.4 Indicator (Autoclave) tape – is a heat sensitive tape that is used to indicate that a specific temperature has been reached during the autoclaving process. Indicator tape undergoes a distinctive color change when autoclaved. Indicator tape confirms only that a specific temperature has been reached during the sterilization process and is not a definitive indicator of sterilization.

4.5 Personal Protective Equipment (PPE) - equipment worn to minimize exposure to hazards that cause injuries and illnesses. PPE may include items such as gloves, safety glasses, face shields,

goggles, safety shoes, earplugs or muffs, hard hats, respirators, protective clothing, coveralls, vests, and full body suits.

4.6 Steam Chemical Integrator (Steam Sterilization Integrator Strip) - a paper or polymeric film which allows steam to penetrate at a certain rate during the autoclaving process. These indicators are designed to monitor all three of the critical variables of the steam sterilization process (i.e., time, temperature and steam).

4.7 Sterilize - to make free from living bacteria, virus, and other microorganisms; the complete destruction of all forms of microbial life, including bacterial and fungal spores.

4.8 User – any authorized laboratory personnel who has successfully completed training on the safe operation of the LabStar 70 Basic Laboratory Autoclave.

5. Procedural Steps

5.1 Training – All users of the autoclave must be trained in the operation of the equipment.

5.1.1 Laboratory personnel wishing to operate the autoclave must successfully complete a training session from laboratory director or SRA.

5.1.2 Training records are kept on file by the Director of Laboratories and Scientific Research Associate. Recertification will occur at three year intervals.

5.2 Equipment Preparation

5.2.1 Ensure the autoclave is empty. If materials have been left in the autoclave, carefully remove and place them on the table next to the autoclave.

5.2.2 Ensure the drain is clear of debris.

5.2.3 Check the door seal and remove any debris with a damp cloth. Inspect the seal for cracks. Notify the laboratory SRA or director of any concerns with the seal.

5.2.4 Turn the autoclave on.

5.3 Material Preparation

Ensure the material is safe for autoclaving. The following table provides a list of autoclave compatible and incompatible items. Consult with the laboratory SRA if further guidance is needed.

Autoclave Compatible	Autoclave Incompatible (Never Put These in the Autoclave)
Glassware (Heat Resistant borosilicate glass, e.g. Pyrex or Kimax)	Acids, bases, and organic solvents
Tissue Culture Flasks	Chlorides and sulphates
Pipette Tips	Chlorine, hypochlorite, bleach
Culture & Media Solutions	Non-stainless steel
Heat Resistant Plastics (Polypropylene, Polycarbonate, PTFE/Teflon)	Polystyrene and polyethylene
Stainless Steel	Polyurethane
Gloves	Any liquid in a sealed container
Bags approved for autoclave	Cracked Glassware

5.3.1 Prepare and package materials appropriately

5.3.1.1 Place all items in a secondary container.

5.3.1.2 Tag each item and/or secondary container with indicator tape.

5.3.1.3 Glassware: loosen lids to prevent pressure buildup, cover all open glassware with foil.

5.3.1.4 Containers of liquid: bottles must not be more than two-thirds (2/3) full, keep 1-2 inches of space between bottles in secondary container, loosen lids to prevent pressure buildup, secondary container should contain about an inch of water.

5.3.1.5 Prepare dry goods and liquids to be autoclaved separately.

5.4 Loading the Autoclave

5.4.1 Required Personal Protective Equipment (PPE): Lab coat, eye protection, heat-insulating gloves and closed-toe shoes.

5.4.2 Place materials in autoclave. Do not autoclave dry goods and liquids in the same cycle.

5.4.3 Do not overload the autoclave. Leave space between items and do not allow anything being autoclaved to touch the sides or top of the chamber

5.4.4 All autoclave pans and containers should be sitting flat and not be angled or leaning inside the autoclave. Do not stack bags on top of each other.

5.4.5 Close the door and wait for the door to latch automatically.

5.5 Operating the Autoclave

5.5.1 Choose the appropriate cycle for your materials and initiate the cycle. If you are unsure of the appropriate cycle consult the laboratory director or SRA. As a general rule:

a. Liquid Cycles: Used when autoclaving liquids (or materials that become liquid at high temperatures) such as broth, media, agar, buffer, saline, and water.

b. Gravity Cycles: Used for autoclaving glassware and other dry goods.

5.5.2 Record information in the Autoclave Log for the load you are processing.

5.5.2.1 Include the date, start time, run number, load volume, load description, cycle duration, operator name, and the result of the integrator test if included.

5.5.2.2 See attachment in Section 6 of this SOP for a blank copy of the Autoclave Log

5.6 Unloading the Autoclave

5.6.1 Personal Protective Equipment (PPE): Lab coat, eye protection or face shield, heat-insulating gloves, and closed-toe shoes.

5.6.2 Ensure the cycle has completed successfully

5.6.2.1 Successful cycle: “program END” will appear on the control screen and the gauges are in a safe range for temperature (<100) and pressure (0psi).

5.6.2.2 Failed cycle: “program was STOPPED” will appear on the control screen and a ⚠ symbol is present in the upper line.

5.6.2.2.1 In the event that the sterilization cycle terminates prematurely (fails), attempt to re-start the cycle or start a new cycle. If the autoclave will not restart or fails another cycle, leave the materials in the autoclave and do not clear the error message. Place an “Out of Service” sign on the autoclave and notify the laboratory director or SRA.

5.6.3 Stand back from the door and carefully open the door no more than 1-2 inches. Allow the autoclave load to stand for at least 10 minutes in the chamber once the door has been opened.

5.6.3.1 Do not agitate containers of super-heated liquids or remove caps before unloading.
5.6.3.2 Do not agitate or remove caps until the liquids have cooled to a safe level.
5.6.4 Wearing PPE, remove items from the autoclave and place them on a stable structure to allow them to cool.
5.6.5 Ensure that the chemical indicator tape lines have changed from white to black. Notify the laboratory director or SRA if the indicator tape does not change.
5.6.6 Record results of run in Autoclave Log.

5.7 Basic Maintenance

5.7.1 Routine maintenance shall be performed according to manufacturers' recommendations.
5.7.2 Refer to the operating manual for detailed instructions on performing maintenance tasks.
5.7.3 Observe the safety instructions *on page 79* of the manual during maintenance work.
5.7.4 Table of basic maintenance tasks and schedule:

Designation	Interval	Manual Reference
Visual inspection of door seal for contamination and wear	Weekly; as needed	*see page 80*
Visual inspection of the product temperature sensor for damage	Weekly	*see page 80*
Chamber cleaning	Weekly	*see page 80*
De-ionized water tank cleaning	Weekly	*see page 81*

5.8 Validating Autoclave Efficacy

5.8.1 There are 3 indicators that are used to monitor the efficacy of the autoclave process:
5.8.1.1 Physical Monitoring Devices: pressure and temperature recording devices built into the autoclave
5.8.1.1.1 Each autoclave has a functional monitoring or measuring device (electronic or dial) to ensure that the recommended temperature is achieved for the proper length of time on each load.
5.8.1.1.2 The LabStar 70 Basic is equipped with electronic monitoring that will automatically indicate a failed cycle if temperature and/or pressure are not maintained for the required amount of time.
5.8.1.1.2.1 If a cycle failure occurs the control screen will display the cycle was not completed successfully and provide information regarding the error or malfunction.
5.8.1.2 Chemical Indicators: indicators that change color after being exposed to specific temperatures and/or steam.
5.8.1.2.1 Autoclave Tape (Heat Indicator): Each item or container sterilized by autoclaving should have autoclave tape attached to it. Autoclave tape confirms that a specific temperature has been reached during the sterilization cycle. Positive verification is noted when black stripes appear on the autoclave tape.

5.8.1.2.2 Steam Chemical Integrator: Every 5th autoclave cycle, the laboratory SRA will include a steam chemical integrator strip with the autoclaved materials.

5.8.1.2.2.1 If the integrator strip dye has migrated completely from the "start" to "finish" zone, proper steam and heat sterilization conditions have been confirmed.

5.8.1.2.2.2 If the dye fails to completely migrate across the strip, the test has failed to demonstrate that sufficient sterilization parameters were generated. The test should be repeated. Replace the steam chemical integrator with an integrator and autoclave again. In the event of a second failed test, place an "Out of Service" sign on the autoclave and begin troubleshooting procedures.

5.8.1.2.2.3 Record the results of the cycle in the Autoclave Log. The Steam Chemical Indicator should be taped to the log next to the cycle record from which it was obtained.

5.8.1.3 Biological Indicator: The biological indicator is the most reliable method to validate autoclave sterilization efficacy.

5.8.1.3.1 The laboratory SRA will test the autoclave with a biological indicator after every 40 hours of operation to validate autoclave performance. The testing procedure is as follows:

5.8.1.3.1.1 Secure a biological indicator test: a vial containing *Geobacillus stearothermophilus* spores.

5.8.1.3.1.2 Place a vial containing spores into a beaker and cover the beaker with aluminum foil. Place the beaker containing the vial into the secondary container holding materials to be sterilized.

5.8.1.3.1.3 Load materials into the autoclave and start the run.

5.8.1.3.1.4 When the cycle has finished and the materials have cooled, retrieve the vial.

5.8.1.3.1.5 Follow manufacturer's directions for testing spore viability.

5.8.1.3.2 Record date, run parameters, autoclave tested, and test results (a sample record sheet is included in Section 6 of this SOP).

5.8.2 **Protocol for handling an autoclave that fails validation with biological indicator:**

1. Remove the autoclave from service. Notify a supervisor.
2. As soon as possible, repeat the biological indicator test in three consecutive cycles. If additional spore tests are positive, the items from the suspect load should be considered nonsterile and be reprocessed. Materials processed since the last acceptable (negative) biological indicator should be recalled/resterilized if possible.
3. Check to ensure that the autoclave was used correctly (for example verify that the correct time and temperature settings were used). If not, repeat using the appropriate settings and recall/reprocess all inadequately processed materials.
4. Check with maintenance support to determine if any irregularities may provide an explanation (electrical for example). Any abnormalities should

be reported to the person who performs sterilizer maintenance.

5. Check to ensure that the correct biological indicator was used, that it was not expired, and that the results were appropriately interpreted. If not, repeat the test using appropriate supplies.
6. If steps 1-5 resolve the problem, and if all three repeat biological indicators from three consecutive autoclave cycles are negative, then put the autoclave back into service.
7. If one or more biological indicators are positive however, do one or more of the following until the problem is resolved:
    a. Request an inspection of the equipment by autoclave maintenance personnel.
    b. Discuss the abnormalities with the autoclave manufacturer.
    c. Repeat the biological indicator tests using a different manufacturer's indicator.
8. If there still is no resolution to the problem, close the autoclave down until the manufacturer can assure that it is operating properly. Retest at that time with biological indicators in three consecutive autoclave cycles.

5.9 Recording and Documentation

5.9.1 Users must maintain records of the cycle/run every time they conduct a sterilization cycle.

5.9.2 The laboratory SRA must keep records of any validation testing performed on the autoclaves.

5.9.3 The laboratory SRA and/or director will maintain records of repairs, service calls, and calibrations of autoclaves.

6. Reports/Charts/Forms/Attachments/Cross References

6.1 Attached: Training certification and sign off form.

Autoclave Training Certification

Autoclave Approved User List

By signing below, I acknowledge that I have read and understood the contents of Burrell SOP on Autoclave Operation, and have agreed to conduct my activities in accordance with Burrell autoclave procedures. I understand that breaching these standards may result in disciplinary action, including termination of my status as an autoclave-approved user. To ensure proper and consistent training, approval is only valid if also signed by the trainer (i.e. the Laboratory Director or Scientific Research Associate).

Date	User Name	User Signature	Trainer Signature

6.2 Attached: Autoclave Log

Autoclave Log

BioScience Research Laboratory Burrell College of Osteopathic Medicine Phone: 575-674-1760 Email: bsrl@bcomnm.org

Building:	200	**Room Number:**	203
Autoclave Manufacturer:	Zirbus	**Autoclave Model/Serial #**	LabStar 70 SN:4559

This form must be completed in its entirety and retained for records.
If extra copies of this form are needed, please contact the Scientific Research Associate or Laboratory Director at bsrl@bcomnm.org.

Date	Load Description	Load Volume	Cycle type or #	Cycle Start Time	Cycle End Time	Cycle Length (min.)	Pressure (mbar)	Temp (°C)	Operator Name	Initials	Steam Chemical Integrator Test PASS / FAIL	

MODIFICATION TO THIS FORM IS STRICTLYPROHIBITED.

6.3 Attached: Validation of Autoclave Performance Testing Record

Validation of Autoclave Performance

Biological Indicator Testing Record

Autoclave Information

Institution: Burrell College of Osteopathic Medicine

Manufacturer: Zirbus

Location: Research Labs 9035 Advancement Ave.

Model: LabStar70 Basic

Building: 200 Room: 203

Serial #: 4559

Name of Tester: _____

Test Conditions

Date: _____

Time: _____

Cycle type: SOLID LIQUID

Cycle duration: _____

Chamber pressure: _____

Chamber temperature: _____

Test Vial Information

Test Vial #: _____

Test Vial type: (e.g. BT Sure) _____

Test Vial lot #: _____

Expiration date: _____

Each Vial contains: (e.g. BT Sure vials contain $2x10^6$ *Geobacillus stearothermophilus* endospores)

_____

Incubation conditions: (e.g. 55°C for 48 hours) _____

Results: _____

Comments

7. Maintenance

Assistant Dean for Research, Director of Research Laboratories, and Scientific Research Associate will review no less than annually.

8. Signature

Signature on File	5/102/2022
Joseph Benoit, PhD	Date

9. Distribution List

Internal/External

10. Revision History

Revision Date	Subsection #	Summary of Changes	New/Cancellation/ Replacement Procedure? (if applicable)	Approval Date
1	Section 6	Remove reference to BCOM and replaced with Burrell College Attached forms have been updated.	Updates and Replaces previous Version	05/10/2022