A GUIDELINE FOR THE SAFE USE OF AUTOCLAVES

NIPISSING UNIVERSITY

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1.0 PURPOSE

The purpose of the “Guideline for the Safe Use of Autoclaves” is to inform potential users and their supervisors of the issues that must be considered to ensure the autoclave process is undertaken in a safe, effective and efficient fashion.

There is currently one autoclave in operation at Nipissing University. A Market Forge Model STME, which is authorized for use by students who have received the appropriate training by the Laboratory and Biosafety Officer (see Appendix 6).

In all cases, there are trained, dedicated technologists who are responsible for ensuring that maintenance, testing and safety practices are followed with regard to the operation of the autoclave. If there are any questions about access to this autoclave, please see the technologist(s) listed in the contacts list beside the autoclaves.

2.0 INTRODUCTION

An autoclave is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of decontaminating or sterilizing the contents of the chamber. Decontamination is defined as the reduction of contamination to a level where it is no longer a hazard to people or the environment, while sterilization is the total destruction of microorganisms present. This is achieved because heat damages the cell’s essential structures, including the cytoplasmic membrane, rendering the cell no longer viable. This will only occur if the material is heated to a specific temperature for a given period of time. These parameters will vary, depending upon the nature of the microorganisms present and the characteristics of the load itself.

To facilitate this transfer of heat, moisture is often added, however this does not guarantee success. A number of other factors must be considered to effect successful decontamination or sterilization. This guideline will outline these in greater detail, as each step in the process is discussed.

3.0 PERSONAL PROTECTIVE EQUIPMENT

Due to the fact that autoclaves utilize steam, heat and pressure, the risk of personal exposure and potential harm is great. The operator must wear the appropriate protective equipment.

Often material to be loaded contains potentially infectious material, so the standard laboratory protective equipment must be worn. This includes:

- Safety eye and face protection (face shield minimizes the risk of facial steam burns when unloading the autoclave),
- Gloves (latex or nitrile gloves prevent contact with contaminated material, while heat resistant, gloves (moisture proof preferred to protect hands from scalding liquids) must be used when loading and unloading the autoclave),
- Lab coats (long sleeves must be used to protect wrists and forearms, plus an apron if a spill hazard exists).

Remember that although the autoclave trays may be cool, the door and walls of the chamber may still be hot enough to cause a burn.

4.0 TRAINING

Training is absolutely required prior to using the autoclave. Not only will this minimize the risk of personnel being harmed, but it is essential to ensuring a successful decontamination or sterilization of your material. Training will also help minimize the risk of damage to the equipment. This guideline is one tool to assist in training. In addition, you must receive training specific to the autoclave you will be using. This will assist you identifying problems with your own load or previous loads, i.e., interpretation of cycle log records. Training will help promote:

- Safety,
- Research quality, and
- Optimal use and care of equipment.

Currently, the Laboratory and Biosafety Officer will ensure that all students, staff and faculty are trained in the use of the autoclave. To receive training, please contact the Laboratory and Biosafety Officer at extension 4180 to arrange a training session.

5.0 ITEMS FOR AUTOCLAVING (THE CAN AND CANNOTS)

Although autoclaving provides an economical way of sterilizing and decontaminating items, not all material can be autoclaved. Some materials present specific hazards when they are autoclaved; such as the generation of toxic/noxious gas. To help you identify what may or may not be autoclaved a general list of items has been included in this guideline.

Items that **CAN** be autoclaved are:

- Cultures and stocks of infectious materials, culture dishes and related devices;
- Discarded live and attenuated vaccines;
- Contaminated solid items such as: petri dishes, disposable pipette tips, pipettes, gloves, paper towel;

- Items for sterilizations such as; glassware, media, aqueous solutions, equipment.

Items that **CAN NOT** be autoclaved are:

- Materials containing: solvents, volatile, chlorinated compounds (HCL, bleach) or corrosive chemicals (such as: phenol, trichloroacetic acid, ether, chloroform) etc.;
- Material contaminated with chemotherapeutic agents;
- Radioactive material (without prior approval);
- Some plastics.

6.0 LOADING AN AUTOCLAVE

This section will address the various steps to be undertaken when preparing and autoclaving the material to be decontaminated or sterilized. The following factors will be discussed: packaging (primary and secondary), identification requirements, and the fundamentals of loading an autoclave to maximize steam penetration. Each of these factors play a critical role in ensuring a successful decontamination /sterilization.

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Prior to Loading an Autoclave:

Verification should be undertaken to ensure the autoclave has been functioning correctly and has been meeting the validation requirements. This verification can be performed by:

1) Reviewing the previous cycle log,
2) Examining the results of the monthly verification records, and
3) Speaking to the individual responsible for the autoclave.

For additional information, please refer to the quality control section of this guideline.

Packaging

As the success of the decontamination/sterilization is dependent upon the penetration of heat, the initial preparation of the material will greatly affect the outcome. Consideration must be given to the primary container (containing the contaminated waste), volume of liquid, amounts of material, and the secondary container (containing the primary container).

The structural integrity of the container is an important consideration. Not all containers can withstand the demands placed on them during the autoclave process. Desirable characteristics are heat resistance, good thermal conductivity, puncture proof and impervious to water.

Good Choices:

- Borosilicate glass (Pyrex) has very low thermo expansion property and therefore resistant to breaking due to heating
- Polypropylene (PP) and polycarbonate (PC) are heat resistant plastics
- Stainless steel is a good heat conductor and thus facilitates sterilization

Poor Choices:

- Polystyrene (PS), polyethylene (PE) and high-density polyethylene (HDPE) do not resist heat well.
- Most containers will identify the type of plastics identified on the base of the container with the appropriate initials imprinted. (PP, PC, PS, PE, HDPE, LDPE...)
- If there is a risk of any material melting, ensure they are placed in a heat resistant secondary container. If in doubt, use secondary containment.

Do not autoclave mixed loads (i.e. waste materials for disposal should not be autoclaved with materials that will be re-used). For example, do not autoclave re-usable glassware, tip boxes, instruments, or media with biohazardous waste. Run two separate loads. This will have the benefit of: preventing re-handling and thereby minimize the risk of contamination, reducing the risk of personal injury due to sorting of sharp objects, and finally avoiding having to separate items that have become stuck together during the autoclaving process.

When packaging the material, consideration must also be given to the final weight of the items, taking into account possible water absorption during the autoclave cycle. Total weight must not exceed the load bearing restrictions of autoclave or cause ergonomic injuries as a result of transferring this material in and out of the autoclave.

The packaging must permit heat (steam) penetration, and ensure pressure differentials are not created, as this will result in breakage (***No sealed containers must ever be placed in an autoclave***). This may be accomplished by using techniques such as:

- Loosening screw caps or using self-venting caps.
- Capping open containers for sterilization with aluminum foil
- Using envelope folds for wrapping Kraft paper or muslin (This has the added benefit of protecting contents from contamination during the opening process.)
- Opening plastic bags slightly prior to loading them into the autoclave.

For bags containing solids, add 250 ml of water (more if the bags are large). This will create additional steam which will displace air from the bag, increasing the rate of heat penetration. It is best to allow the water to drain down the sides of the bag, taking care to avoid splashes and/or spillage.

Primary Container:

The primary container is the container which comes into direct contact with the contaminated material of fluid. This may include such items as: flasks or vials holding liquids (either media or infectious material), wrapping paper or muslin protecting instruments, and biohazard bags containing waste.

Containers for liquids (± agar):

Only borosilicate glass (Pyrex™ or Kimax™) can be used to hold liquids since they can withstand the temperature changes. There should be no visible defects (chips or cracks) in the glass. Regular (flint) glass is not stable and can crack when it is cooling with disastrous consequences for a person holding that vessel. Any glass that holds liquids must have a Pyrex™ or Kimax™ label. Acid or solvent bottles, old pickle jars should never be used for liquids.
In addition to using the right glass, always put material into a metal tray so that the user can handle the trays rather than grabbing the actual flask. The trays also contain any spills to avoid having to clean out the autoclave.

Volume should be less than half full to avoid boiling over of the media as the pressure is released and vessels must not be sealed: use a foam plug or paper or tin foil cap. Do not use a stopper or have a threaded lid that is tight to avoid a build-up of pressure inside the vessel. Threaded lids must sit loose on the vessel and when the load is removed, tighten the lid to avoid drawing unsterilized air into the container as the liquid cools (once boiling has stopped).

Containers for solids:

Make sure they can withstand the temperature of the autoclave (121C), especially for plastic containers (bags, plastic lids, etc.). Consult a Nalgene™ catalogue that has a table of the chemical and thermal resistance properties of a variety of different materials. Also, it may be that an autoclave bag is rated at 125C for steam heat but be aware that after the steam is released from the chamber, the walls stay hot because there is steam in the jacket around the chamber. Thus, after the cycle is over, the bag is exposed to dry heat as long as they sit inside the autoclave and we believe this is when the bags can melt. Remove all bags within 2 hours; don't leave them overnight as they may melt. Always place bags in a metal or Nalgene tray to contain any leaks or melting plastic.

Empty solid bottle containers

There are two traditional methods for enhancing the sterilization of solid bottom containers such as glass bottles, pans, etc. in gravity displacement cycles. These are:

1. Place 1 mL of water for each liter of volume in the bottom of each container. The expansion of the water into steam, as the product is heated, forces most of the air out of the object, thus allowing steam to reach all surfaces and effect sterilization.
2. The better, more reliable method is to orient all objects in a manner which would allow air to flow out (i.e. on their side or inverted). When the steam enters the chamber, it tends to layer over the air. When inverted, however, the object is oriented so air can flow out. As air flows out of the container, it is replaced by steam. Steam can now reach all surfaces, and effect sterilization.

Bagged Biohazardous Waste:

Biohazardous bags are purchased in two sizes. One small sized bag that fits the containers for the students work benches. These are loosely tied and stored in a large red bin at the back of the lab and is marked “biohazardous waste to be autoclaved” this bag is lined with a large red biohazardous bag. Both bags are clearly marked as biohazardous waste.

Again, always use a metal tray under the bags of waste in case there is a leak (hole in the bag or bag meltdown). Don't overfill the bag as the top should be open when placed in the chamber.

Do not overfill the autoclave chamber. A maximum of three biohazardous bags should be autoclaved in one 60-minute cycle. Steam must get into the bag from the top and must be able circulate around the outside to effectively heat the material. Fold edges down slightly to keep bag open wide.

Volumes/Amounts

As volume and density will impact the heat transfer and steam penetration it is important not to fill the containers beyond the 50-75% of the holding capacity. This also allows for liquid expansion, preventing overflow. Similarly with solid material, the additional available volume will allow the contents to shift during transfer into a secondary container or the autoclave without spilling out of the bag. Avoid packing or compressing the contents to achieve volume limits as this will restrict steam penetration.

Secondary Containers

The sides of the secondary container must be sufficiently high to contain any spill that may occur. It is recommended that secondary containers of both 15 cm and 30 cm be available. Extreme care must be taken to ensure they are capable of withstanding the demands of the

autoclave procedure. Age of pans can often cause them to become brittle or to soften when heated. Regular inspection of the secondary container will help identify compromised containers. In addition, they should be made of material that facilitates the transfer of heat to compensate for the restricted airflow within the chamber caused by their presences. This becomes much more a factor with the 30 cm high containers.

It is necessary to label all material being placed in the autoclave. This is important for the following reasons:

1) So the material may be subsequently sorted and returned to the appropriate laboratories;
2) Should a problem or concern arise regarding the function of the autoclave the individuals potentially affected may be informed, and;
3) Should a spill occur the risk may be assessed based on the contents involved. Remember you may not be aware of a problem until much later in your research project, for example: contaminated cell cultures.

Therefore the following information must be affixed:

- Name of responsible person;
- Contact information;
- Identification of contents (including volume of liquid contained).

Use of Temperature Sensitive Tape and Steam indicators:

Temperature sensitive tape can be affixed to bags and each individual item. When this tape has been exposed to high temperatures lines will appear. Thus it may be used to indicate that the labeled material has been autoclaved. *It is not proof that the autoclave cycle was successful at decontaminating or sterilizing the contents***.** A biological indicator or other means must be used to act as a quality control validator.

Similarly, the steam indicator strips are useful to indicate the autoclave produced steam.

Transporting Packaged Material to the Autoclave

When transporting material to be autoclaved, use a cart with guard rails. Ensure the use of a secondary container to collect any spillage should the cart be knocked or jarred during transport. Use a direct but not heavily populated route. Surface decontaminate the container prior to transport, unless there is no risk of contamination.

Fundamentals of Loading to Ensure Success

As much attention must be applied to loading the autoclave, as was given to packaging. Again, the determining factor is ensuring heat/steam penetration. Therefore, care must be given to avoid overloading the chamber, placing bags in the chamber which are too large, or adding too much weight which will tax the design elements of the autoclave. Consideration must also be given to ergonomic factors.

Simple measures can be used to ease the flow of heat and steam thorough and into the contents of the containers. Here are some fundamental rules:

- Load the material in such a fashion to present the least resistant passage of air exchange through the load, from the top of the chamber to the bottom.
- Avoid crowding or stacking.
- For solid loads, use a perforated metal pan to allow steam penetration and to avoid the formation of air pockets.
- Place packages on their edges to enhance steam penetration, place a rack or other item against these items to prevent them from slipping.
- For loads which are mixed (fabric and hard goods) place the hard goods on the bottom to prevent possible condensation from dripping on to the fabric.
- Place empty flasks, test tubes or other non-porous containers on their sides with loose covers. This provides a horizontal pathway and prevents trapping air pockets.
- Ensure containers do not touch each other, this will ensure all surfaces are sterilized.
- No items should touch the top or sides of the autoclave container as the container is pushed inside.
- Liquids and dry goods are processed separately as they require different cycle selections.
- A load of liquid filled containers should be of similar size, shape, content and volume; because exposure time is based on these characteristics.
- Run material to be sterilized separate from those to be decontaminated.

7.0 OPERATING AN AUTOCLAVE

Factors to Consider

Each autoclave will have specific instruction for its own use. It is important to follow the manufactures recommendation and each user must receive hands-on training on its use. Although many factors must be considered when determining the length of time required some general guidelines can be provided.

Factors that will affect your cycle times will be:

- If the autoclave will be used for sterilization or decontamination
- Manufacturers recommendation for media sterilization
- If the material is primarily solid or liquid
- Volume of liquid
- Shape and size of containers used
- Thermo conductivity properties of the container
- Viscosity of the liquid
- Density of the material
- Position of the load within the autoclave chamber. To be effective the autoclave must reach and maintain a temperature of 121-123 $^{\circ}$C for at least 30 minutes. This is achieved by using saturated steam under at least 15 psi of pressure.

It is important to recognize the length of time required to achieve this temperature will be dependent upon the factors stated above. In addition, the sensing device which records this temperature is not located within the load of material being autoclaved. Therefore, additional time may be required to ensure the centre of the load has achieved this temperature, for example with samples that have a very high biological load the contact time must be extended to minutes.

Determining the Correct Cycle Time

As the cycle time will vary with the composition of the load, it is important to determine the appropriate time requirement. Assuming the minimal time of 30 minutes, may prove to be a very costly mistake. It is up to the user to ensure that the load put through has met the conditions for sterilization. Therefore, it is imperative that the autoclave be tested for efficiency of sterilization (see Quality Control section below for more information).

Cycle Failure

If the autoclave cycle fails to be completed, the load must be autoclaved again. If there was a failure the orange light will be on to indicate the autoclave did not complete. In the past this has only occurred when the water level inside was insufficient.

8.0 UNLOADING AN AUTOCLAVE

The greatest risk of personal injury occurs during the process of unloading the autoclave. Not only is the risk of burns or scalding significant, but one may also be exposed to the vapours and gases generated by the inadvertent autoclaving of volatile chemicals. Super-heated liquids also pose of risk of exploding if they are shaken or moved during the cooling process. In addition, glassware can break if the autoclave door is opened too quickly, and sufficient time is not

provided for them to approach room temperature. *Consequently, extreme caution must occur during this final stage*.

- Procedures to follow:
- Wear all necessary personal protective equipment (heat resistant gloves, eye and face protection, lab coat, apron if handling liquids).
- The chamber pressure gauge of the autoclave should be zero before opening the autoclave's door.
- Crack door slightly and stand back to allow steam to escape. To minimize the risk of accidents caused by steam escape, the person who opens the autoclave door should stand beside the opening rather than in front of the opening.
- Slowly open autoclave door. Opening the autoclave door too quickly may result in glassware breakage and/or steam burns to the skin.
- If boiling or bubbling is present, wait until it subsides. Assess the risk of super-heated liquids.
- Over exposure of saline or water is not a critical factor (as it is with media), so these liquids may be allowed to cool (for 10 min.) in the autoclave after cracking the door to release the steam. This is also recommended for all other items.
- Bring the trolley to the chamber.
- Using heat resistant gloves carefully transfer the containers (pans) to the trolley. Be careful not to jolt the containers as this could result in breakage.
- Move the containers to a draft free area.
- If not already cool, wait 10 minutes prior to storing sterilized material or preparing autoclaved waste for disposal.
- After every use, it is advised to close the autoclave door but do not seal the door as this will shorten the life span of the rubber gaskets on the door.
- Verify that the steam strip indicators have shaded to indicate steam was achieved throughout the autoclave load. The paper strips are economical and therefore if autoclaving three biohazardous bags it would be helpful to put one steam indicator in each bag.
- The container that could be contaminated by a liquid splash (due to boil over) or by direct contact with contents of the waste bag (such as melted agar) should always be washed after each use if you are certain the autoclave run was an effective run, as indicated by a steam strip indicator.

9.0 DISPOSING OF AUTOCLAVED WASTE

Completion of an autoclave cycle is insufficient information to determine that the biohazardous waste is no longer a biohazard. We need indicators to assess the autoclave run produced steam *throughout* the load. The indicators used in our facility are biological indicators and steam indicators. A steam indicator indicates the autoclave cycle produced steam. A biological indicator contains spores and therefore are a more accurate test to determine if the steam and heat cycle was sufficient to kill pathogens known to withstand high heat. When a biological indicator has been included in the biohazardous waste, the biohazardous waste must be stored in a holding bin for 48 hours allowing the biological indicators to develop and to indicate negative growth (effectively sterilized) or positive growth result (ineffective sterilization). Nipissing University's Level 2 lab has 4 large biohazardous bins marked as either; 1) storage before autoclaving or 2) storage of autoclaved waste awaiting BI results. Once testing is complete and indicates negative growth, the BI test log book is filled out to record the results.

Once proven to be waste with no biohazard present, it is now important to alter the hazard awareness signage and symbols to reflect this. The autoclaved waste bags should be placed inside a plastic trash bag (black) designed for domestic applications. It is greatly recommended to place only one or two biohazardous bags inside a single plastic trash bag (domestic applications) to prevent an overload and to minimize the risk of breakage. Ensure the black bag is securely tied close. Maintenance staff will not pick up a red biohazardous bag so ensure the black garbage bag does not have the red bags poking out.

If the biological steam indicator came back as a positive result, the waste would need to be put through another autoclave cycle appropriate for the volume.

10.0 PREVENTATIVE MAINTENANCE

Preventative maintenance will greatly increase the efficiency of your autoclave, reduce downtime and save costly repairs. It may be considered as a scheduled inspection, which could result in minor adjustments or repairs, major repairs, or premature replacement of parts. It is designed to maintain safety, and delay or avoid an emergency.

The work associated with this activity is greatly reduced when: the autoclave is used properly, the loads are packaged and loaded in an appropriate fashion (to minimize the risk of spills and

explosions, and material touching the chamber walls), and secondary containers are used to collect spills.

Daily / Weekly Maintenance Activity:

Although the equipment specific manual will provide specific instructions, some general guidelines can be provided. Steps that should be taken daily or weekly, depending upon use pattern and control during packaging and loading, are:

1. Disinfect exterior surfaces of the autoclave;
2. Check door gasket for wear;
3. Clean interior walls with tri-sodium phosphate, other mild detergent or as recommended by the manufacturer. Never use a strong abrasive or steel wool. Rinse with tap water after cleaning;
4. Check primary and secondary containers for integrity (stress fractures, cracks, chips etc.);
5. Consult manual to determine other recommended activities.

Yearly Maintenance Activity:

A semi-annual inspection and maintenance program should be undertaken only by qualified individuals.

11.0 QUALITY CONTROL

A number of tools are available to assess the performance of the autoclave; these include physical, chemical and biological indicators. It is important to note that these indicators will only respond to time, temperature and moisture conditions, and not to organic load.

Physical Indicators:

Pressure and temperature recording devices such as thermocouples can be placed inside the load to determine the temperature achieved in the bag itself.

Chemical Indicators:

These indicators change colour after being exposed to specific temperatures, for example the steam indicator paper strips. Upon exposure to the given temperature the change will occur; it is not time related. Therefore these indicators can only attest to the temperature attained and not to exposure time and hence success of sterilization.

Biological Indicators:

Biological indicators are used in the efficacy testing of the autoclave process to effectively sterilize the contents being treated. *Geobacillus stearothermophilus* spores are used, as they are the most resistant organism to steam autoclaving. The standard for testing[1] requires a 6 log10 reduction of spores to ensure a level 4 treatment. This means the spore ampule must contain a 10^6 population of spores, and those spores must be killed by the steam or heat treatment. Since spore vial populations come in more than one population density, it is important to purchase the correct test vials.

To determine the effectiveness of the autoclave process the biological indicator must be placed in a typical test load (solid or liquid) and exposed to the typical cycle conditions. This is the standard method of validating the effectiveness of your autoclave procedures. *Testing using a biological indicator must be undertaken at least every six days of operation[2] or once every two weeks, whichever time is less. The testing regime should alternate between solid load verification and liquid load verification.*

Solid Load Sterility Verification:

1. Read and follow the suppliers' instructions.
2. Place *G. stearothermophilus* in centre of representative test load.
3. Process load in normal fashion
4. Extract and incubate the ampule of *G. stearothermophilus* as instructed by the manufacturer.
5. Use another ampoule (same lot #) not autoclaved to act as a positive control.
6. Check for colour change at regular intervals during the incubation period (8, 12, 24, and 48 hours).
    a. If media is yellow and turbid the autoclave process has FAILED. Immediately upon noting yellow colouration, re-run all samples with new biological indicators.
7. If failure continues to be noted, either increase the time of exposure or initiate repairs to the autoclave.
    a. Note the autoclave cannot be used again until validations procedure indicates that autoclave is now adequately sterilizing the material.
8. Record all results. (Positive and Negative)

[1] Guideline C17: Non-incineration Technologies for Treatment of Biomedical Waste (Procedures for Microbiological Testing, Ontario Ministry of Environment, October 2002, p. 10.
[2] Ibid.

Liquid Load Sterility Verification

1. Obtain a media bottle of the sizes that would normally be used for media.
2. Tie a string to an ampule of *G. stearothermophilus* and then to a stir bar. Make sure the string length between the stir bar and ampule will allow the ampule to be suspended in the middle of the bottle contents (see illustration to the right).
3. Fill the bottle with an appropriate volume of water and loosely cap the bottle.
4. Place the bottle in the middle of a typical liquid load and process in the normal fashion.
5. Extract and incubate the ampule as instructed by the manufacturer.
6. Use a second unprocessed ampule from the same lot number to act as a positive control.
7. Check for colour change at regular intervals during the incubation period (8, 12, 24, and 48 hours).
    a. If media is yellow and turbid the autoclave process has FAILED. Immediately upon noting yellow colouration, re-run all samples with new biological indicators.
8. If failure continues to be noted, either increase the time of exposure or initiate repairs to the autoclave.
    a. Note the autoclave cannot be used again until validations procedure indicates that autoclave is now adequately sterilizing the material.
9. Record all results (positive and negative).

13.0 RECORD KEEPING

Records are a vital component of the autoclave process. These records act as historical proof that the autoclave has been meeting the regulatory requirements and/or industrial standards. Autoclave testing records and the printed records of the sterilization cycles must be kept for a minimum period of two years and must be in a format that is available for inspection by the Ministry of Environment staff[3].

Three types of records are to be kept:

1. **Autoclave Log**
    - Identifies the users, nature of the load, cycles used and exposure times (See Appendix 1).
2. **Validation Records**

[3] Guideline C-4: The Management of Biomedical Waste in Ontario, November 2009, p. 13

- Record the results of the validation activities undertaken. This would include the results of the six day use biological indicator (Appendix 2).
    - Biological Indicators (Appendix 2)
        - Record biological indicator information: brand, lot #, expiry date.
        - Record the date, operator, cycle time, cycle temperature of the test run.
        - Record results: colour change noted (Failure), no colour change-yellow (Pass)

3. **Performance Records**
    - Records the dates that problems were encountered, remedial action taken and any service calls required. (See Appendix 4) In addition, annual service reports should be kept on file for the life of the equipment[4].
    - This record will permit a general assessment of the condition of the autoclave; and helps to answer the question "Should we be considering replacing this autoclave in the next 3-5 years?"

14.0 MISTAKES IN AUTOCLAVING

A number of events (operator and mechanical) can cause the autoclave cycle to fail, but most tend to be directly related to the packaging and loading consideration taken. This may result in having to re-autoclave material, or modify the cycle conditions (length of exposure time or temperature).

Unfortunately, lack of diligence during the autoclave process can be very costly in terms of:

- Personal injury;
- Down time;
- Lost experimental data;
- Expensive and/or lengthy repairs;
- Inappropriate disposal to landfill resulting in regulatory violation and an inquiry/fine.

Typical mistakes:

- Insufficient personal protective equipment (resulting in injury).
- Rushing (resulting in burns, spills etc.).
- Inappropriate material selection (resulting in glassware breakage or unexpected or undesired melting of containers).

[4] Guideline C17: Non-incineration Technologies for Treatment of Biomedical Waste (Procedures for Microbiological Testing, Ontario Ministry of Environment, October 2002, p. 12.

- Sealing containers (resulting in pressure build-up, explosions, and lack of steam penetration).
- Lack of use of secondary containers (resulting in the chamber becoming contaminated).
- Over-filling containers (resulting in liquids boiling over and loss containment of solids).
- Poor loading practices (resulting in lack of steam penetration through the load).

14.0 REFERENCES

- A guideline for the safe use of autoclaves, University of Ottawa, 2003.
- Laboratory Biosafety Manual, 2nd Edition, World Health Organization, Geneva
- "Using a Gravity Displacement Steam Autoclave in a Biomedical Laboratory" video produced by School of Public Health, University of Minnesota under contract with National Institute of Allergy and Infectious Disease
- Cooney, Timothy E., *Techniques For Steam Sterilizing Laboratory Waste*, AMSCO Research and Development
- Autoclave Procedures, Office of Radiation, Chemical and Biological Safety, Michigan State University www.orcbs.msu.edu/biological/autoclave/sterilization.htm
- Safety Net - Effective Use of Autoclaves, University of California - Davis, Environmental Health and Safety, University of California www-ehs.ucdavis.edu/safetynet/effective-use-autoclaves
- Biological Safety Update, UCSF Autoclave Quality Control Program, University of California San Francisco, Environmental Health and Safety www.ehs.ucsf.edu/Manuals/BSMEntireDoc.htm
- Medical Waste Management Program, University of California - Riverside, Environmental Health and Safety www.csuchico.edu/ehs/mwm.htm
- "Procedures for Disposal of Biomedical Waste at UWO", University of Western Ontario
- Guideline C-4: The Management of Biomedical Waste in Ontario, Ontario Ministry of Environment.
- Guideline C-17: Non-incineration Technologies for Treatment of Biomedical Waste (Procedures for Microbiological Testing), Ontario Ministry of Environment.

Appendix 1 – Autoclave Operation Log

100 College Drive, North Bay, ON P1B 8L7
Autoclave location: H220
Year _____

Biological Research Materials Only
Steam disinfection/autoclave record

Date	Description of Contents / Notes	Cycle Selected	Temperature Achieved (°C)	Sterilize time (min.)	Printed Name / Signature

Appendix 2 - Biological Indicator Test Results

Room Number: H220 **Autoclave Model**: Market Forge Model STM-E

Contact Person(s): Sarah Minnery, Amy Stillar, Ashley Marcellus.

Testing using a biological indicator (BI) must be undertaken at least every six days of operation or once every two weeks, whichever time is less. The testing regime should alternate between solid load verification and liquid load verification.

Date of Autoclaving and Incubation	Person conducting test	Test Load Type (Solid or Liquid)	Cycle Selected and Temperature	Cycle Time	BI Brand Lot Number and Expiry Date	Incubation Time (Hours)	Results Pass or Fail

Note: All tests done at 121ºC and 15.5 psi.

BI = Biological Indicator (*G. sterothermophilus*); Slow = liquid cycle; Fast = instrument cycle; F = front; M = middle; B = back

Appendix 3 - Autoclave Performance Record

Room Number: H220 **Autoclave Model**: Market Forge Model STM-E

Date	Problem	Remedial Action	Comments	Description of Service Call

Document Revision History

Date	Author	Revision
May 30, 2011	Dave Vadnais	New document.
July 11, 2011	Dave Vadnais	Added equipment specific instructions (Market Forge and Steris).
Sept 2014	D. Vadnais	Removed equipment specific instructions for Steris due to being permanently out of service.
Sept 2015	D. Vadnais	Minor updates throughout the document.
Mar 14, 2016	D. Vadnais	Minor updates throughout the document.
Mar 24, 2022	M. Banks	Minor updates throughout the document.
Mar 28, 2022	S.Minnery	Updates throughout the document