**Biosafety Program: Standard Operating Procedure**

Autoclave Maintenance, Validation and Verification

**(Revised February 2024)**

**Purpose:** The purpose of an autoclave maintenance, validation and verification program is to ensure that the autoclave operates as designed, reaches its established program parameters for sterilizing and/or decontamination to ensure confidence that loads that must be sterilized prior to disposal are in fact sterile. Autoclaves can be technically complicated and steam sterilization does have limitation to their efficacy. As autoclaves are essentially small pressure vessels used at very high temperatures, they can, if not functioning properly also represent a hazard.

**1.0 Annual Maintenance**: Autoclaves will be subjected to an annual inspection and preventative maintenance which will cover:

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| --- | --- | --- | --- |
| INSPECT SOLENOID VALVES |  |  | FLUSH & DESCALE  |
| INSPECT MANUAL VALES |  |  | INSPECT LEVEL CONTROLS |
| INSPECT PNEUMATIC VALVES |  | INSPECT PRESSURE CONTROLS |
| INSPECT CHECK VALVES |  |  | INSPECT PRESSURE CUT OUT |
| INSPECT FLOW CONTROLS |  |  | INSPECT LEVEL PROBES |
| INSPECT PRESSURE REGULATOR |  | INSPECT SIGHT GLASS VALVES |
| INSPECT RELIEF VALVES |  |  | INSPECT SOLENOID VALVES |
| INSPECT TEMPERATURE PROBES |  | INSPECT MANUAL VALVES |
| INSPECT PRESSURE TRANSDUCERS |  | INSPECT RELIEF VALVES |
| CHECK TEMPERATURE READINGS |  | INSPECT PUMP & MOTOR |
| CHECK PRESSURE READINGS |  | INSPECT RELAYS & CONTRACTORS |
| INSPECT CH. DR. STRAINER |  |  | INSPECT FUSES & HOLDERS |
| INSPECT HEAT EXCHANGER |  |  | INSPECT WIRING |  |
| INSPECT WATER EJECTOR |  |  | INSPECT HEATER CONNECTIONS |
| TEST FOR VACUUM LEAKS |  |  |  |  |  |
| INSPECT PIPING ASS'Y FOR LEAKS |  |  |  |  |
| INSPECT COND. DOOR GASKET |  |  |  |  |
| INSPECT RADIAL ARM DOOR ASS'Y |  |  |  |  |
| INSPECT & LUBE DOOR GUIDES |  |  |  |  |
| & TRACKS |  |  |  |  |  |  |
| CHECK DOOR LOCK |  |  |  |  |  |
| INSPECT STEAM TRAPS |  |  |  |  |  |
| RUN TEST CYCLES TO VERIFY |  |  |  |  |
| CYCLE PARAMETERS & PHASES |  |  |  |  |

**2.0 Autoclave Validation**: Autoclave validation is intended to test the efficacy of the decontamination process under challenging conditions. Using a representative load, which consists of the maximum quantity of material of a particular type of load, the autoclave is tested and in conjunction with the use of Bioindicators (BI), chemical integrators (CI) and the autoclave data output, the autoclave and autoclave method tested are validated as successfully capable of decontamination. All autoclaves will be validated annually following the annual inspection and servicing or following any repair service which might have impacted the autoclave’s ability to meet run parameters. Validation will be conducted and results recorded by the Biosafety Officer (BSO) or their designate.

**Validation method (to be performed by BSO or designate):**

**Loading**

* A representative load will be a collection of mixed waste consisting of autoclave bag, paper towels, gloves, petri dishes and culture devices to a maximum of 3 Kg (6.6 lbs).
* A bio-indicator (typically *Geobacillus stearothermophilus*) will be inserted into a perforated copper tube which will be inserted into the middle of the load. A second bio-indicator will be identified and left as a positive control i.e. non-autoclaved bio-indicator.
* The bioindicators used will be the 3M Attest 1262. Bioindicators used for validation will be of the same lot number and have the same expiry date.
* The bag will be loaded into the autoclave and a program for solid waste (121oC for 30 minutes, Pre-vac) will be run.

 **Upon completion of the run:**

* The validator will check to ensure that the program parameters (temperature, pressure and run time) were met from the printed copy of the run.
* While wearing gloves, remove the copper tube from the load and extract the Bioindicator (caution the tube may still be hot). Place the empty copper tube back in its holder.
* Verify the chemical indicator on the vial changed from rose to brown. If the indicator did not change colour, but the test parameters, from the autoclave readout/printout, indicated it met the program parameters, re-run the load again with new bio-indicators.
* If the chemical indicator does not change colour a second time, either reduce the load size, ensure it has been loaded into the autoclave properly or arrange for technical service of the autoclave.
* If the chemical indicator does change colour, proceed to incubating the bioindicators as per the 3 M Attest 1262 (Figure 2.3) instructions below.

**Incubation of indicators:**

* Following the run, the bio-indicator is removed from the load and incubated (in the supplied 3 M incubator) along with the positive control as per the directions for the bio-indicator (see below for 3M Attest 1262 instructions). Following 48 h of incubation in the 3M incubator the vials will be assessed.
* If the test vial (the one in the autoclave) is purple and the positive control is yellow following incubation, then the load was sterilized and validation was successful.
* Should both the test vial and positive control vial be yellow following incubation, then the load was not sterilized, and the load should be repeated with a longer run time with new bio-indicators.
* Should the bio-indicators indicate a failure on the second run, either reduce the load size, ensure the autoclave was loaded properly or if necessary, arrange for technical service of the autoclave. The BSO should be made aware of any failure during a validation trial.
* Should both the test vial and the positive control vial be purple upon 48 hours of incubation then the spores were likely not viable, or the control vial was incubated improperly. Re-test with a newer batch of bio-indicators.

Upon successful completion of the validation test (colour change on chemical indicator and test vial is purple with positive control vial yellow upon incubation) the Biosafety Office (BSO) will complete the autoclave validation certificate. The certificate will be posted on each autoclave (Appendix 1)

**3.0 Load Verification Procedure to be Followed by All Research/Teaching Personnel/Graduate & Under-Graduate Students**

Load verification is the periodic testing of a decontamination process (autoclaving) to detect process or equipment failure. At Trent University, verification testing is mandatory for all waste loads which contain biohazardous (Risk Group (RG) 1,2 or 3) material.

**Verification of a Solid Waste Load:** Any load where sterilization is used for decontamination of biohazardous agents will require verification. For solid or mixed loads, a Bioindicator (such as a 3M Attest 1262) or a 3M Attest Steam Chemical Integrator 1243, Type 5 can be used. Users are welcome to use load verification for other purposes as well, but it is not mandatory.

**Procedure for solid load verification using a Biological Indicator (BI).**

 At Trent, only 3M Attest 1262 bioindicators are acceptable.

* Select two bioindicator vials from your stock. Ensure the Lot # on both vials are the same and that you are still within the expiry date on the vial. Label one vial with the date and indicate it is the “load” vial. Label the second vial with the date and indicate “control”
* Insert the “load” bioindicator into the copper tube with wire attached. Set the “control” indicator aside for now.
* While wearing the protective gloves, insert the copper tube into the load so that the bottom of the tube is approximately in the middle of the load. Ensure the retrieval wire/sting extends out the top of the bag. Try to avoid having the tube surrounded by soft plastics as they may melt during autoclaving and make it difficult to remove the tube from the load or the vial from the tube.
* Fasten a strip of chemical indicator tape to the outside of the bag.
* Using a waterproof, indelible marker, label the outside of the bag with your name and the building and lab location from which the waste originated (e.g., ESB A 203).
* Load the bag into the secondary container and place it into the autoclave chamber.
* Use the correct autoclave program with the correct exposure time (which is the amount of time the autoclave will maintain 121o C at 15 psi above ambient atmospheric pressure, normally a minimum of 30 minutes is required but larger loads may require more).
* Run the load.
* When the run has finished and you have verified on the printout that the autoclave reached 121 o C and stayed there for the programmed exposure time, and the load has cooled sufficiently to be safe to handle, remove the load from the autoclave and verify the strip of chemical indicator on the outside of the bag, changed colour. If the chemical indicator does not change colour, you will need to re-run the load likely with a different exposure time.
* If the chemical indicator tape changed colour, while wearing protective gloves, remove the copper tube with the bioindicator by pulling on the wire. “Caution” the tube may still be hot. Remove the indicator by tipping the copper tube. Place the copper tube in the plastic container labelled “contaminated copper tube holder” until the bioindicator has been incubated and sterility confirmed.
* Verify the chemical indicator on the outside of the bioindicator changed colour (from rose to brown) indicating it reached the autoclave set temperature (121o C). If the bioindicator chemical indicator did not change colour then you will need to re-run the load. Check the load volume, density and ensure the top was open to allow steam to penetrate the load. You may also need to lengthen the exposure time. You will need to replace the bioindicator with another from the same Lot # and re-run the load.
* If the chemical indicator on the outside of the bioindicator changed colour (indicating the outside of the tube reached the set temperature then, incubate test indicator with the positive control as per the bio-indicator and incubator instructions (Figure 2). Remember that until the indicator is incubated and the results known, you should treat the waste as potentially still contaminated.
* While incubating the bioindicators, ensure the bag is closed and placed in the secured container labelled for autoclave waste. Ensure the “Do Not Dispose” orange label is facing out on the container. Do not dispose of the waste until the results of the bioindicator incubation (48 h) clearly indicate that sterility of the *G.* *stearothermophilus* was achieved. Remember that at this point while we would expect sterility to have been achieved, it is not yet verified, so treat the waste as still contaminated and wear the appropriate protective equipment.
* Once incubated for 48 hours, and if the bio-indicators shows sterility was achieved (as per the description below) then the bag (load) can be disposed of through normal landfill procedures. Flip the sign on the secured container to “Ready for Disposal” green label facing out. Complete the autoclave log book and indicate the bio-indicator test was performed and the outcome (Pass/Fail). Remove the copper tube from the plastic holding container as it can now be considered clean. The copper tube should be left near the autoclave for use by others.
* If the bio-indicators did not verify a positive sterility for the load, verify autoclave program parameters were correct, exposure time was sufficient (if necessary extend the exposure time), proper loading of the autoclave (including the quantity and type of waste) and re-run the load with new bioindicators. If the verification fails a second time, record in the autoclave log and contact the Biosafety Officer.
* Put the unautoclaved (control) bioindicator in your next load of biohazardous waste—it will need to be autoclaved to inactivate the contents prior to disposal.

Note: Whenever you are handling material which has not been confirmed as “sterile” you must treat it as being contaminated. Proper procedures and the use of personal protective equipment (PPE) must be followed.

**Verification of a solid load using a Steam Chemical Integrator (SCI).**

At Trent only an Attest 1243, Type 5 steam integrator is allowed. The 3M™ Attest™ Steam Chemical Integrator, 1243A/1243B, is an ISO 11140-1:2014 Type 5 Integrating Indicator for use in determining whether the process conditions necessary for sterilization were met inside each pack. The Type 5 Chemical Integrator monitors time, temperature and steam and provides fast and easy to read results.

Procedures for load verification using 3M Attest 1243 type 5 steam integrator.

1. Ensure the waste load is properly prepped for autoclaving.
2. Tape or attach a 3M™ Attest™ Steam Chemical Integrator 1243A/1243B to the outside of the bag or container which contains the Biohazardous waste. Do not place the integrator in a location where water may accumulate.
3. Process the load according to established autoclave procedures.
4. After processing, the dark color should have entered the green ACCEPT window of the 3M™ Attest™ Steam Chemical Integrator 1243A/1243B. If the dark color has not entered the green ACCEPT window, a red REJECT result is indicated and the items in the pack, peel pouch, container system, or tray were not exposed to sufficient steam sterilization conditions. These items should be returned for reprocessing. (see Figure 3).

**Verification of Liquid Loads:**

Verification of liquid loads of biohazardous waste requires the use of a bio-indicator designed to be immersed into the middle of the liquid. (Figure 1). Verification of autoclaved liquid loads is required only for high volume material (greater than 500 mL). Liquid loads of < 500 mL should not have exposure times of less than 30 mins as there is a “lag” time involved to heat the liquid to 121 o C. Samples of 500 mL of water should take approximately 30 minutes to heat up to temperature and therefore exposure times would be 30 min plus sterilization time.

Liquid loads of biohazardous material greater than 500 mL require the use of an immersible bio-indicator such as the Sterikon Plus tm Bio-indicator. The ampoule contains *Geobacillus stearothermophilus* and nutrient agar. The thermal resistance is such that the spores are totally killed after 15 minutes when heated to a temperature of 121oC in an aqueous liquid. After being autoclaved, the ampoules are incubated (with a positive control) for 48 hours at 60oC. A clear, purple colour of the liquid in the ampoule after incubation indicates sterility was achieved. A yellow orange colour with some turbidity indicates that sterilization was not achieved. (Follow the instructions for the test material you purchase).



Figure 1: Sterikon plus Bioindicator for liquid biohazardous waste.

**Method for Liquid Verification:**

* Tie a string or use a small gauge wire, around the neck of the bio indicator. Suspend the bioindicator into the middle of the liquid in the container. If your load has multiple containers, pick the container which will be in the “middle” of the load and suspend a bioindicator in that one container. Keep another bioindicator outside the autoclave as a positive control.
* Using the chemical indicator tape, attach the string or wire to the outside of the container.
* Loosely cover the opening of the container with either aluminum foil or a cap. Ensure the opening is not sealed so that the pressure can equalize during the run.
* Place container(s) of liquid(s) into the secondary container and load the autoclave chamber.
* Select the appropriate liquid cycle and verify the exposure time in the program will be long enough to account for the lag time and the sterility time.
* Run the autoclave.
* Once complete, let the containers cool down prior to handling. Ensure the chemical indicator tape changed colour indicating the outside of the container reached 121 o C. If the chemical indicator did not change colour the load will need to be re- run likely with a longer exposure time.
* When safe to do so remove the bioindicator and following the instructions for the bioindicator test, incubate the test and positive control vials as per the kit instructions.
* During the incubation and once the containers are cooled, the containers should be closed and kept in a secure location until the results of the bioindicator test are known.
* If, following incubation, the bioindicators indicate sterility was achieved the material and containers can be disposed of.
* If the bioindicators indicate sterility was not achieved, then the load will need to be re-run with either fewer containers (less volume) or a longer exposure time. New bioindicators will be required.
* Complete the log sheets fully.



Figure 2. 3M Attest 1262 Instructions



Figure 3. 3M Attest 1243, Type 5 Steam Integrator interpretation instructions.

Autoclave Verification and Use Log: (**example only, the actual log may differ in appearance but will be in a binder near the autoclave).**

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| --- | --- | --- | --- | --- | --- |
| **Date of run****dd/mm/yyyy** | **Time of run****hh:mm am/pm** | **Supervisor and Lab of Origin****(e.g.****Waterson, CSB 109)** | **Name of Person operating autoclave****(e.g., Calvin Hobbes)** | **Load type****Check one** | **Run verification**1.Check all indicating sterility,2. If not biohazardous a waste load) use N/A.3. Note: chemical and/or bioindicators mandatory for decontamination of biohazardous waste |
|  |  |  |  | **Solid waste** | **Liquid****waste** | **Solids** | **liquids** | **Run Parameters achieved?****(Yes or No)** | **Chemical indicator colour change****(P=Pass, F=Fail)** | BI or SCI shows load sterility(P=Pass, F=Fail) |
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Autoclave Annual Validation Certificate

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| --- | --- |
| **Autoclave Make and Model:** |  |
| **Building and Room #:** |  |
| **Date of Validation Test (dd/mm/yr):** |  |
| **Test Parameters: 121 oC, 30 minutes exposure time, representative solid waste** | Details: |
| **Results of Chemical Indicator (Pass/Fail)** |  |
| **Result of Run Parameters (from printout):** |  |
| **Bio indicator Lot # and Expiry (Ensure Control and Test BI are the same Lot and Expiry)** | **Lot # : Expiry Date:** |
| **Results of Bioindicator (48 h incubation) (Pass or Fail)** |  |
| **This Autoclave has met its Validation Requirements:** | Yes | **This autoclave has not met its Validation Requirement:** |  No |
| **Biosafety Officer name (print first/last):** |  |
| **Biosafety Officer signature:** |  |
| This copy must be retained for 7 seven years (or as deemed necessary by BSO) |