

Bovey 4th floor Tissue Culture and Autoclave Facilities Standard Operating Procedures

If you are using the autoclave, read only the autoclave SOP

If you are using the autoclave and the tissue culture labs, read both the autoclave SOP and the tissue culture labs SOP

Bovey 4th floor Autoclave Standard Operating Procedures

Covid-19 Prevention Measures.

Self-Assessment

- **Do not come to work if you are feeling unwell. If you have any COVID-19 symptoms, self-isolate, contact a health care provider and inform your supervisor.**
- If you become sick while at work, stop working, isolate yourself from others, inform your Supervisor and go home as soon as possible. Take measures to protect others around you and avoid public transportation.
- If in the past 14 days you have been in close contact with a person suspected or confirmed to have COVID-19, do not come to work and inform your supervisor immediately.
- If you have tested positive for COVID-19, contact Occupational Health and Wellness (OHW) at ohw@uoguelph.ca or extension 52647. OHW will follow the guidance and directions from Public Health.

Hygiene

- **All users are required to wear a face covering (mask). The mask should cover the mouth and nose, and be free of any defects. Guidance for using non - medical face masks is available at: <https://www.uoguelph.ca/hr/covid-19-guidance-using-non-medical-face-masks>**
- Please wash hands upon entering the autoclave room before putting on gloves. Wash your hands with soap and water for the recommended 20-30 second interval with water as warm as you can tolerate. The soap is located by the sink in the autoclave room.
- Avoid touching your face, eyes, nose, and mouth.
- Before exiting the lab, wash your hands with soap and water as described above.

Sanitizing Surfaces

- Wipe down the control panel, valve handles and door handles of the autoclaves with 70% ethanol before and after use.
- There is a wash bottle labelled 70% ethanol on the bench to the right of the sink. Cheese cloth wipes are in a box on the shelf at the right of the sink.
- Please contact Bob Nichols when the supply of ethanol or wipes becomes low.

Social Distancing

- In order to maintain the recommended 2 meter distance between individuals there should not be more than one person working in the autoclave room at any given time.
- If you unexpectedly encounter an individual, allow that person to pass while maintaining the 2 meter distance
- Please reserve the autoclaves and/or space in the autoclave room using the calendar that was shared on OneDrive. Contact Bob Nichols (bnichols@uoguelph.ca) or Mukund Shukla (mshukla@uoguelph.ca) to obtain a link to the calendar.

Instructions for Operating the Autoclaves

For fire or medical emergency call extension 52000

No one is allowed to work alone in the tissue culture facilities outside of regular working hours. If you have to be in the lab outside of working hours, you must have someone with you. That person should be familiar with the Bovey building and the tissue culture facilities.

There are two Amasco Eagle series 3021 autoclaves located in the tissue culture lab (room 4219) of the Bovey building.

Wipe down the control panel, valve handles, and door handles of the autoclaves with 70% ethanol before and after use.

To turn on the autoclave:

1) Turn on the power switch located behind the printer panel door then open the water supply valve and the steam supply valve which are found behind the upper access panel. **Do not** adjust the Hi-Lo valve which is also located behind the upper access panel. The Lo setting provides a sterilization temperature of 121 °C which is adequate for our applications.

2) Two types of sterilization cycles are commonly used: **gravity** and **liquid**. A gravity cycle is used to autoclave dry materials, and a liquid cycle is used when autoclaving liquid materials.

3) The autoclave can be programmed to run four sterilization cycles. The first three cycles have been preset as follows:

Cycle 1	liquid 20 minutes
Cycle 2	liquid 45 minutes
Cycle 3	gravity 20 minutes, dry time 0 minutes

You should not attempt to change these 3 cycles. If a different sterilization cycle is required, you can change cycle 4.

To change Cycle 4:

- 1) Lift up the sliding panel located below the display window to reveal the touch pad.
- 2) Press and release the CHANGE VALUES pad. The digital display will read “DO YOU KNOW ACCESS CODE” Press and release the CHANGE VALUES pad again. This should take you directly to cycle 4.
- 3) View the available cycles (gravity, liquid, flash) by pressing the VALUE (up down) arrows on the touch pad. Release the arrow when the desired cycle is displayed. This will select the desired type of cycle along with the default settings (sterilize temperature, sterilize time, dry time) for that type of cycle.
- 4) If you wish to change the default settings, move to the item you wish to change with the cursor arrows (left or right). Increase or decrease the displayed setting with the VALUE (up down) arrows. Repeat until all desired changes have been made, then press save values. The cycle you programmed should now be displayed when you press cycle 4.

Preparation of materials to be autoclaved:

- 1) Only autoclavable materials may be placed in the autoclave. If you don't know if something is autoclavable, check with the manufacturer.
- 2) All materials to be autoclaved must be placed in an autoclavable tray. The use of trays will prevent liquids from spilling into the autoclave in the event of a boil-over or broken bottle.
- 3) Liquids to be autoclaved must be in a vessel that is at least twice as large as the volume to be autoclaved (i.e. if you are autoclaving 500 ml of media, put it in a bottle or flask that will hold at least 1 litre).
- 4) Caps must be slightly loose so that pressure created during the cycle does not cause the vessel to break. For screw cap containers, tighten and then loosen the lid by one-half turn.
- 5) Use the heavy duty media bottles (pyrex) whenever possible. They are sturdier than other glass bottles and therefore less likely to break in the autoclave.
- 6) Contaminated tissue culture plates, bacterial plates, fungal plates and plant material should all be placed inside an autoclavable Biohazard bag for autoclaving. The bag should be sealed, placed in an autoclavable tray and autoclaved for 45 minutes on the liquid cycle.
- 7) The 45 minute cycle (cycle 2), should be used when autoclaving contaminated material or when autoclaving a large number of 'sterile water' bottles.

Running a cycle:

- 1) Place the tray containing the materials to be autoclaved in the autoclave. Gently close the door and turn the handle clockwise to tighten.
- 2) Select the desired cycle by pressing the corresponding number then press the number a second time within 5 seconds to begin the cycle.
- 3) The autoclave will progress through the cycle automatically as follows:
 - purge
 - charge
 - sterilize
 - exhaust (fast for gravity cycle, slow for liquid cycle)
 - dry (if selected for gravity cycle)
 - complete (buzzer will sound)
- 4) You must remain in the autoclave room until you are sure that the autoclave is functioning properly. (i.e. the autoclave is running at the proper temperature and pressure, the sterilisation step of the cycle has started, the door is closed tightly and there is no steam leaking out around the edge of the door). If there are any malfunctions press the reset button and contact the lab supervisor.

Removing materials from the autoclave:

- 1) **Caution:** at the end of the liquid cycle, the digital display on the autoclave will read: **“WARNING HOT LIQUIDS OPEN DOOR 1 INCH WAIT 10 MIN”**. This means that you should wait for the full 10 minutes before removing your liquids. A sudden full opening of the door or removal of liquids before the 10 minute wait period is over may cause the liquids to boil over or the bottles to break. **This could result in serious injury.**
- 2) After the 10 minute wait period is over the buzzer will sound. Open door and check your materials. **Do not move bottles if any boiling or bubbling is present.** If no boiling or bubbling is present, you can remove your materials. Be sure to wear the proper heat resistant gloves. Avoid sudden jarring or striking of one bottle against another.
- 3) If you have any spills in the autoclave you must report them to the lab supervisor immediately. It is your responsibility to clean up any mess that you have caused.
- 4) If you have any problems or malfunctions with the autoclave, you must:
 - a) Report them to the lab supervisor immediately.
 - b) Note the problem and the date on the autoclave malfunction sheet located on the white board located outside of the autoclave room.

5) Autoclaved contaminated waste or biohazard waste must be removed from the autoclave room promptly.

6) If the cycle that you are using is appropriate, add any items that have been left on the “To be autoclaved” table to your load. (i.e. Do not add liquid items to a gravity cycle).

7) If you must remove other people’s material from the autoclave, place the autoclaved material on the lab bench to the right of the sink. “Sterile water bottles” for the TC lab use are the exception. If you are removing a load of sterile water bottles, tighten the lids of all the bottles and place them in the sterile water bottle cabinet in room 4215 or on a cart near the sterile water bottle cabinet.

8) Autoclaves must be turned off at the end of the day.

Trainer _____ Trainee _____
Date _____

Bovey 4th floor Tissue Culture labs Standard Operating Procedures

Covid-19 Prevention Measures

Self-Assessment:

- **Do not come to work if you are feeling unwell. If you have any COVID 19 symptoms, self-isolate, contact a health care provider and inform your supervisor.**
- If you become sick while at work, stop working, isolate yourself from others, inform your Supervisor and go home as soon as possible. Take measures to protect others around you and avoid public transportation.
- If in the past 14 days you have been in close contact with a person suspected or confirmed to have COVID-19, do not come to work and inform your supervisor immediately.
- If you have tested positive for COVID-19, contact Occupational Health and Wellness (OHW) at ohw@uoguelph.ca or extension 52647. OHW will follow the guidance and directions from Public Health.

Hygiene

- **All users are required to wear a face covering (mask) AT ALL TIMES while working in the tissue culture facilities. The face mask should cover the mouth and nose, and be free of any defects. Guidance for using non - medical face masks is available at: <https://www.uoguelph.ca/hr/covid-19-guidance-using-non-medical-face-masks>**
- Please wash your hands upon entering the tissue culture facility before putting on gloves. Using the sinks in the media preparation and containment rooms, please wash your hands with soap and water for the recommended 20-30 second interval with water as warm as you can tolerate. The soap is by the sinks in both of these rooms.
- Avoid touching your face, eyes, nose, and mouth.
- Before exiting the lab, wash your hands with soap and water as described above.

Sanitizing Surfaces

- Wipe down all door and cabinet handles, light switches, faucet handles and fridge/freezer door handles with 70% ethanol. Wash bottles labelled 70% ethanol and cheese cloth wipes are located on the shelves above the benches in the media preparation room and on

the shelf in the containment room and transfer room. The Office of Research phase-in guidelines recommend that these surfaces be disinfected at the beginning and end of the day, or, if researchers are working in shifts, at the beginning and end of every shift.

- All shared equipment, including pH meters, media dispensers, pipettors, balances, carts, stir plates and microscopes should have user interface surfaces cleaned with 70% ethanol before and after use.
- There are wash bottles labelled 70% ethanol and cheese cloth wipes on the shelves above the benches in the media preparation room and on the shelves in the transfer room and containment room. Please contact Bob Nichols when the supply of ethanol or wipes becomes low.

Physical Distancing

- In order to maintain the recommended two meter distance between individuals, the number of people working in any one space at the same time should be limited to the following:

Room 4218 (Transfer room) - 4 people.

DO NOT work in flow benches that have been taped off to allow for physical distancing. Only use flow benches that have been marked for use.

Room 4217 (Media lab) - 2 people.

Two media preparation stations have been set up with the required equipment and are marked for use. In order to maintain 2 meter of physical distancing, **prepare media on the side of the bench that has been marked for use.**

Do not work on the opposite/adjacent side of the bench while someone is preparing media.

Room 4219 (Autoclave room) - 1 person

Room 4216 (Containment room) - 1 person

- Please email Bob Nichols to reserve space in the tissue culture facility rooms. The calendar for the next week will be posted on OneDrive on Thursday of each week. Please have your space requests in by Wednesday at 4:30 pm; reservations will be managed in a manner that is fair and equitable for all labs. If you have booked space for a certain time but find that you no longer need it, please cancel the booking so the space will be available to others. Contact Bob Nichols (rnichols@uoguelph.ca) or Mukund Shukla (mshukla@uoguelph.ca) to obtain a link to the calendar.

- If you encounter an individual while moving from room to room, allow that person to pass while maintaining the 2 meter distance.

General Safety

- **For fire or medical emergency call extension 52000**
- Safety Data Sheets for the chemicals are kept on the shelves above the first bench in the media preparation room. You are required to be aware of any hazards associated with the chemicals that you are using prior to use and take the appropriate measures to ensure the safety of yourself and others.
- Chemical Spill Kits are located at the end of the first bench in the media preparation lab.
- The biohazard spill kit is in the cupboard above the sink in the containment room
- The first aid kit is located at the end of the first bench in the media preparation room. If you remove any items from the first aid kit, notify the lab manager that the items need to be replaced.
- Eye Wash stations are located at the sink in the media preparation room and containment room.
- A safety Shower is located outside of the lab in the hall across from the entrance to the tissue culture facilities
- Fire extinguishers are located at the doors of the rooms in the tissue culture facility.
- Closed-toed, closed-heeled shoes constructed of a resistant material (preferably leather) are required while in working in the lab. Sandals are not permitted. High-heeled shoes are strongly discouraged as they increase the potential for tripping or falling. Long pants to the ankle are required. Shorts, skirts or dresses are not permitted
- Gloves should be worn when working with chemical or biological hazards. Each research group is responsible for providing their staff with gloves.
- Do not wear gloves outside the laboratory
- No one is allowed to work alone in the Tissue Culture labs outside of regular working hours. If you have to be in the lab outside of working hours, you must have someone with you. That person should be familiar with the tissue culture facilities and the Bovey building.
- Lab doors must be locked if you are working outside of working hours.
- Food and drinks are not allowed in the lab.
- Ensure that you have completed the required safety training

Media Room

- Two media preparation stations have been set up with the required equipment. There is one station per bench. Do not work on the opposite (adjacent) side of the bench while

someone is preparing media. Media preparation stations for use are separated by 2 meter and are marked for use with signage.

- This room is for media preparation only. Do not bring plant material into this room.
- All lab users are responsible for a clean lab. Clean workspace and equipment immediately after use. Do not leave materials on the benches, carts, or floors. Put them away.
- Wash all labware and culture vessels properly then rinse well (at least 3 x) with deionized water.
- Do not leave anything in the sink, not even for a moment.
- Put the clean items away when they have dried.
- The balances are only to be used for media preparation. Clean any spills on the balance or bench after each use.
- If you are heating media to melt the gelling agent (or for any other reason) make sure the flask that you are heating is not more than 2/3 full.
- Clean any spills on the hot plate/stirrer. Wait for hotplate to cool to a safe temperature before attempting to clean. Do not use chemical cleaners.
- If you spill something on the bench, Remove the benchcoat and replace it with new benchcoat.
- The chemicals are arranged on the shelves in alphabetical order. Make sure that they stay in that order when you put them back.
- The fridges/freezers are used to store chemicals and solutions required for media preparation or cryo-experiments. Media should be stored in the fridges only if necessary. Do not store cultures or samples in these fridges.
- Report all accidents or breakages to the lab manager.
- Notify the lab manager when you notice chemicals or any other lab supplies are getting low so more can be ordered. Do not wait until we have run out.

Transfer Room

- Some of the flow benches have been taped off to allow for physical distancing. Do not work in these hoods.
- This room is for sterile plant cultures only. Do not open or work with fungal cultures, bacterial cultures or contaminated plant cultures in this room.
- Open flames are not allowed. Bead sterilizers are available.
- Do not leave the flow bench unattended for extended periods of time.
- Do not leave material in the hoods or on the carts overnight. When you have finished your work at the hood, you should remove all materials from the hood and the cart and put them away.
- The cart and hood should be cleaned with 70% ethanol after use.
- Garbage bags containing plant material and /or media should be taken to the dumpster at the end of the day.

Containment Room

- Open flames are not allowed. Bead sterilizers and sterile disposable loops and spreaders are available.
- The biosafety cabinet is equipped with a UV light. Turn the light on and leave it on for 10 minutes, then turn it off prior to wiping the interior surface of the cabinet with 70% ethanol.
- When you have finished your work, remove all of your materials from the cabinet and wipe it down with 70% ethanol.

General Items

- Personal projects are not allowed in the lab. Do not bring plant material or cultures that are not part of the official research program into the lab.
- Do not remove equipment, chemicals or supplies from the labs without permission.
- Each room should be set up with the necessary equipment for the work done in that room. The equipment should not be taken out of one room to do work in another room. For example, the pipettors in the media preparation lab are for media preparation only. They should stay in the media preparation lab. They should not be taken do work in any other room.
- The carts in the tissue culture area are for tissue culture work only. Do not take them to the greenhouse, containment room, molecular lab or any other lab.
- Avoid coming from the greenhouse to the lab. If you have to work in the greenhouse, try to arrange your schedule so that the greenhouse work is done after the lab work.

Trainer_____

Trainee_____

Date_____