1. PURPOSE

1.1. To ensure the safety of people in the Microbiology laboratories at Thompson Rivers University (TRU).

2. SCOPE

2.1. This procedure applies to all employees and students at TRU.

3. PRECAUTIONS

POTENTIAL HEALTH & SAFETY HAZARDS

HAZARD		TO PROTECT YOURSELF
Fire		Know the location of the extinguishers. Have no open flames in the lab. Do not keep volatile solvents in open beakers.
EXPLOSION		Never heat a closed system
CHEMICAL OR THERMAL BURN		Many inorganic chemicals are corrosive to the skin and eyes. Many organic chemicals are corrosive. Always assume hot plates are hot
LACERATION		Lubricate rubber stoppers before trying to force onto glass. Use gentle pressure with rotation on the glass part.
ABSORPTION OF CHEMICALS		Keep chemicals off the skin. Organic substances are absorbed through the skin even if they do not burn or are corrosive. Repeated exposure may result in contact dermatitis. Ensure any gloves worn do not have holes in them before using them.
INGESTION OF CHEMICALS		Do not use mouth suction for pipettes. Wash hands before handling anything going into your mouth. Do not eat or drink in the lab. Do not store food or drink in the lab.
INHALATION OF CHEMICALS		Do <u>not</u> *sniff* a product to establish what it is. Many common solvents are toxic if inhaled in any quantity or over a period of time. Use the fume hood.
EYE INJURY Chemical splashes		Use the appropriate protective eyewear. Chemicals may splash into eyes during pouring or use – wear chemical splash goggles and full-face shield.

4. ASSOCIATED DOCUMENTATION

Doc. Number	Doc. Title
	Lab Risk Assessment and Control Form
	Training Records
	Student Competency Checklist
	Incident Investigation Form

5. PROCEDURES AND RESPONSIBILITIES

GENERAL LABORATORY SAFETY PRACTICES

5.1. Laboratory doors should be kept closed. People must be advised of potential hazards before entering the work area.

5.2. Safety glasses must be worn in all designated laboratory areas.

5.3. Lab coats must be worn in the laboratory areas by all personnel, including visitors, trainees and others entering or working in the laboratory. Contaminated clothing must be disinfected by appropriate means.

5.4. Mouth pipetting is strictly prohibited.

5.5. Eating, drinking, smoking, chewing gum and/or storing food is not permitted in the laboratory areas.

5.6. The lab should be kept clean and free of materials not pertinent to the work.

5.7. Work surfaces should be decontaminated at least once a day and after any spill.

5.8. Employees must wash their hands after handling infectious materials and before leaving the laboratory.

5.9. Gloves must be worn for all procedures that involve contact with body fluids, infectious materials, or infected animals. Gloves must be autoclaved.

5.10. All spills, accidents and possible exposures to infectious materials must be reported immediately to the Biosafety Officer.

5.11. The Lab Supervisor will ensure that training in laboratory safety for infectious materials is provided.

5.12. All procedures should be performed carefully to minimize the creation of aerosols.

5.13. All contaminated or infectious liquid or solid materials must be decontaminated before disposal or re-use.

5.14. Where infectious agents are used in a laboratory, a biohazard warning sign incorporating the universal biohazard symbol must be posted on the access door to the work area.

PERSONAL PROTECTIVE EQUIPMENT (PPE)

5.15. The type and extent of clothing and equipment to be selected for any particular procedure depend on the research operations and levels of risk associated with them. At a minimum, a lab coat, closed-toe shoes, and gloves must be worn in any microbiology laboratory. Lab coats, closed-toe shoes, and gloves prevent bio-hazardous materials from contact with the skin, including areas where there might be cuts, abrasions, or dermatitis. The legs are a vulnerable area if uncovered, so it is inappropriate to wear skirts or shorts. Closed-toe shoes protect the feet from spills as well as injuries from dropped sharps.

5.16. Lab Coat:

5.16.1. A lab coat protects street clothing from contamination and prevents possible cross-contamination from any normal flora present on the skin.

5.16.2. Lab coats must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory.

5.16.3. Coats must be properly fastened.

5.16.4. If contaminated, lab coats should be decontaminated by autoclaving before being placed in the laundry. If decontamination is not possible, any contaminated coat should be placed in the biohazard waste container.

5.17. Gloves:

5.17.1. Appropriate gloves must be worn for all procedures that might involve direct or accidental skin contact with bio-hazardous materials. Latex or vinyl gloves offer a high level of dexterity and a higher level of sensitivity; however, they don't offer a great deal of protection from needle sticks, animal bites or sharps. All gloves will eventually permeate and should therefore be changed periodically. If gloves become contaminated or torn, remove immediately and wash hands with soap.

5.17.2. Some procedures may require double gloving.

5.17.3. Gloves should overwrap the cuff and lower sleeve of the lab coat.

5.17.4. Gloves must be removed prior to leaving the laboratory and decontaminated with other laboratory wastes before disposal.

TYPE	ADVANTAGES	DISADVANTAGES	FOR USE WITH:
Natural rubber latex	Low cost, good physical properties, dexterity	Poor against oils, greases, organic solvents, ethidium bromide. May cause allergic reactions.	Bases, acids, alcohols, dilute aqueous solutions. Fair vs. aldehydes, ketones.
Natural rubber blends	Low cost, dexterity, generally better chemical resistance than natural rubber.	Physical properties often inferior to natural rubber. May cause allergic reaction.	Bases, acids, alcohols, dilute aqueous solutions. Fair vs. aldehydes, ketones.
Polyvinyl chloride (PVC)	Low cost, very good physical properties, average chemical resistance.	Plasticizers can be stripped.	Strong acids and bases, salts, aqueous solutions, alcohols, oils, greases and petroleum products.
Neoprene	Average cost, average chemical resistance, average physical properties, high tensile strength, high heat resistance.	Poor vs. chlorinated hydrocarbons	Oxidizing acids, alcohols, anilines, phenol, glycol ethers, solvents, oils, mild corrosives
Nitrile	Low cost, excellent physical properties, dexterity	Poor vs. chlorinated organic solvents	Oils, greases, aliphatic hydrocarbons, xylene, perchloroethylene, trichloroethane, ethidium bromide. Fair vs. toluene.
Butyl	Good resistance to polar organics, high resistance to gas and water vapour	Expensive, poor vs. hydrocarbons, chlorinated solvents	Glycol ethers, ketones, esters, aldehydes, polar organic solvents
Polyvinyl alcohol (PVA)	Resists broad range of organics, good physical properties.	Very expensive. Water sensitive, poor vs. light alcohols, acids and bases.	Aliphatic and aromatic hydrocarbons, chlorinated solvents, ketones (except acetone), esters, ethers
Fluro-elastomer (Vitron®)	Good resistance to organic and aromatic solvents. Flexible.	Extremely expensive. Poor physical properties. Poor vs. some ketones, esters, amines	Aromatics and aliphatic hydrocarbons, chlorinated solvents, oils, lubricants, mineral acids, alcohols.
Norfoil, Silver Shield™, 4H™	Excellent chemical resistance.	Poor fit, stiff, easily punctures, poor grip.	Use for Hazmat work. Good for range of solvents, acids and bases.

CULTIVATION OF BACTERIA ON LABORATORY MEDIA

Inoculation of Culture Media

5.18. For microbiological investigations it is essential to learn the skills of inoculating specimens onto culture media:

5.18.1. Always practice aseptic technique,

5.18.2. Clean bench-top with supplied disinfectant before beginning your work and upon completion,

5.18.3. Ensure loops and picks are flamed upon completion of you work, and

5.18.4. Discard any waste bio-hazardous material in the appropriate area, to ensure adequate disinfection is completed.

6. EQUIPMENT

CENTRIFUGES

6.1.1. Safe use of centrifuges requires proper maintenance and operation. Failed mechanical parts or improper operation can result in release of projectiles, hazardous chemicals and bio-hazardous aerosols. Maintenance and repairs must be performed only by trained, qualified personnel. To maintain your safety, sample integrity and the equipment, follow these guidelines.

6.1.2. Ensure that centrifuges have an interlocking device that will prevent both the lid from being opened when the rotor is in motion and the centrifuge from starting when the lid is open.

6.1.3. Ensure that centrifuge tubes are free of hairline cracks, stress lines and chipped rims prior to use.

6.1.4. Ensure that tube materials are chosen such that they provide the necessary chemical resistance and speed rating.

6.1.5. Avoid over-filling tubes.

6.1.6. Cap or stopper centrifuge tubes.

6.1.7. Use sealed centrifuge buckets (safety cups) or rotors that can be loaded and unloaded in a biological safety cabinet or chemical fume hood as appropriate.

6.1.8. Decontaminate the outside of the cups/buckets and rotors before and after centrifugation.

6.1.9. Inspect O-rings regularly and replace if they are cracked or dry.

6.1.10. Ensure that the centrifuge is properly balanced. Load the rotor with samples arranged symmetrically. Opposing tubes must be of equal weight. If necessary, use "water blank" tubes to balance sample tubes of unequal weigh. Do not use sight or volume to conclude that tubes are balanced. Use an electronic balance to balance tube before using them in an ultracentrifuge.

6.1.11. Ensure that the prescribed speed limitations of the rotor or centrifuge are never exceeded.

6.1.12. Unless fitted with a suitable exhaust system, do not centrifuge materials capable of creating flammable or explosive vapours.

6.1.13. Remain with the centrifuge until it has reached its programmed speed.

6.1.14. Abort the run immediately if you hear abnormal vibration, whining or grinding noises. Check the rotor lid and balance.

6.1.15. At the end of the run ensure that the rotor and centrifuge are cleaned according to manufacturer's instructions. Never use abrasive cleaners.

6.1.16. Rotors are easily damaged. Never use metal tools to remove tubes or clean the rotors.

6.1.17. If the centrifuge is connected to a vacuum pump, ensure that the pump exhaust is connected to a trap.

6.1.18. If bio-hazardous materials are being centrifuged and the centrifuge is connected to a vacuum pump, ensure that a HEPA filter is installed between the centrifuge and the vacuum pump.

BLENDERS, GRINDERS AND SONICATORS

6.1.19. When used with infectious agents, mixing equipment such as shakers, blenders, grinders, sonicators and homogenizers can release significant amounts of hazardous aerosols, and should be operated inside a biological safety cabinet whenever possible.

6.1.20. Ensure equipment has safety features that will minimize leaking and prevent operation if blades are exposed.

6.1.21. Ensure that any equipment that could move during use is secured to a bench or the floor as applicable.

6.1.22. Ensure equipment is in good condition prior to use.

6.1.23. Allow aerosols to settle for at least one minute before opening containers.

6.1.24. Do not use flammable solvents in equipment such as blenders and stirrers as

they can also produce a large amount of flammable vapours.

ELECTROPHORESIS

6.1.25. The use of voltages of approximately 200 V and currents of more than 80 mA in electrophoresis procedures could create the potential for an electrical shock if the equipment is not operated properly.

6.1.26. Use physical barriers to prevent inadvertent contact with the equipment.

6.1.27. Ensure that electrophoresis equipment is properly grounded.

6.1.28. Inspect electrophoresis equipment regularly for damage and potential buffer tank leaks.

6.1.29. Locate equipment away from high traffic areas and away from wet areas such as sinks or washing apparatus.

6.1.30. Use of ground fault circuit interrupters is recommended.

6.1.31. Display warning signs to identify the electrical hazards (i.e. “Danger – High Voltage”).

6.1.32. Turn off power before connecting leads, opening the lid or reaching into the chamber.

6.1.33. Ensure that lead connectors are insulated.

GAS CHROMATOGRAPHS

6.1.34. Gas chromatography (GC) procedures involve the use of compressed gas cylinders and may involve the use of flammable solvents and toxic chemicals. Be familiar with the use and handling of compressed gas cylinders, and with hazardous properties, precautionary measures, and handling instructions for any hazardous materials being used. Refer to MSDSs (found on-line using the MSDSonline icon on the desk top) or other reliable reference material. The following guidelines will assist in the safe operation of GCs.

6.1.35. Wear proper eye protection. GC columns are fragile and breakage could result in small projectiles during handling. As well, samples are prepared in various hazardous solvents that could damage the eyes upon contact.

6.1.36. When cutting a GC column, be sure that the cut is made away from the body.

6.1.37. Ensure that GC column cutters are capped or otherwise stored to prevent injury when not in use.

6.1.38. Discard small pieces of GC columns as sharps waste.

6.1.39. Ensure that the oven is allowed to cool before installing or removing a column or injector or prior to performing maintenance.

6.1.40. Ensure that gases are turned off prior to removing or installing a column.

6.1.41. Test for leaks after the installation of the column and whenever a leak is suspected. Use a technique that will not damage or sacrifice the integrity of the instrument.

6.1.42. Electron capture detectors (ECD) have a radioactive source and therefore need to be registered as part of the University's Radiation Safety program. ECDs may not be relocated or discarded without permission of the Radiation Safety Officer. Contact the Radiation Safety Officer at extension 2874 for more information about Canadian Nuclear Safety Commission (CNSC) requirements.

6.1.43. Ensure that the instrument and gases are turned off and the power cord disconnected prior to performing maintenance.

GLASSWARE

6.1.44. Improper use of glassware can lead to injuries in the laboratory. The following guidelines will help in the safe use of glassware.

6.1.45. Use only the right size and type of glassware for any given operation.

6.1.46. Ensure that glassware is in good condition prior to use (i.e. no cracks, chips, significant scratches).

6.1.47. Discard broken glassware in appropriate containers.

6.1.48. Cut glass tubes/tubing by scoring using a file or equivalent. Cover the glass with a piece of cloth and break at the score over a piece of cloth/paper to catch any pieces.

6.1.49. Wear leather or other cut-resistant gloves when inserting glass tubing into a stopper or flexible tubing. Fire polish tubing ends and lubricate glass to make the connection easier. Ensure that stopper holes are appropriately sized and carefully insert tubing by carefully twisting back and forth.

6.1.50. Wear leather gloves when removing glass tubing from flexible tubing or a stopper. If difficult, carefully cut with a scalpel blade or other appropriate glass cutter. Ensure that cuts are made away from the body.

6.1.51. Ensure glassware is stored away from the edges of benches so that it cannot easily be knocked.

HEATING BATHS

6.1.52. Heating baths are designed to heat materials to constant temperature. They may be filled with a variety of materials including water, mineral oil, sand, glycerin, paraffin or silicone oils, depending on the bath temperature required. The following are precautions for heating baths:

- locate on a stable surface, away from flammable and combustible materials including wood and paper,
- ensure liquid has cooled before moving the heating bath,
- do not fill over the "full mark",
- ensure baths are equipped with controls that will turn off the power if the temperature exceeds a preset limit,
- ensure that the thermostat is set well below the flash point of the heating liquid in use,
- equip the bath with a non-mercury thermometer to allow a visual check of the bath temperature, and
- take care not to allow water to get into oil baths as violent splattering may result.

6.1.53. Steams baths are often safe alternatives for heating because they provide a consistent temperature that will not exceed 100°C. However, care must be taken to prevent scalding due to dermal exposure to the steam or steam lines.

6.1.54. Water baths are the most common heating bath found in the laboratory. When using a water bath:

- clean the bath regularly; a disinfectant can be added to the water,
- decontamination can be performed by raising the temperature to 90oC or higher for 30 minutes once a week, and
- unplug the unit before filling or emptying.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHS

6.1.55. High performance liquid chromatography (HPLC) procedures often require handling of flammable and toxic solvents. Refer to MSDSs (found on-line using the MSDSonline icon on the desk top) or other reliable reference material. The following guidelines will assist in the safe operation of HPLCs.

6.1.56. Wear appropriate eye protection. Since the HPLC is operated at high pressures,

it is possible for fittings to fail, resulting in a sudden release of solvent.

6.1.57. Use "elephant trunk" ventilating system above fraction collectors, especially with normal phase HPLC.

6.1.58. Inspect and empty the waste containers as required.

6.1.59. Ensure that waste collection vessels are vented.

6.1.60. Ensure secondary containment of waste containers.

6.1.61. Never clean a flow cell by forcing solvents through a syringe: syringes under pressure can leak or rupture, resulting in sudden release of syringe contents.

6.1.62. High voltage and internal moving parts are present in the pump and auto sampler. Switch off the electrical power and disconnect the power cord when performing routine maintenance.

OVENS, HOT PLATES AND HEATING MANTLES

6.1.63. Ovens are commonly used in the lab to evaporate water from samples, provide a stable elevated environment and to dry glassware. Heating mantles are used to heat reaction or sample solutions in round-bottom flasks or reaction vessels, and hot plates are used to heat various general laboratory solutions. Bunsen burners may be used only after obtaining approval from the supervisor. The following precautions should be followed to ensure safe use.

6.1.64. Ensure that laboratory ovens and hot plates are designed to prevent contact between flammable vapours and heating elements/spark-producing components.

6.1.65. Avoid heating toxic, even mildly volatile materials in an oven unless it is continuously vented outdoors.

6.1.66. Glassware that has been rinsed with an organic solvent is to be rinsed with distilled water or equivalent before being placed in an oven for drying.

6.1.67. Hot plates or ovens whose thermostat fails must be removed from service until repaired. Heating devices whose temperature rises above that required could create significant fire hazards.

6.1.68. Heating mantles must be used in conjunction with a variable autotransformer; care must be taken not to surpass the maximum voltage of the mantle recommended by the manufacturer.

6.1.69. Discontinue use of any heating mantle where the heating elements have become exposed.

ULTRAVIOLET LAMPS

6.1.70. Exposure to ultraviolet light (UV) may result in serious and painful injury to the eyes or skin depending on the wavelength and intensity of the light and the duration of exposure.

6.1.71. Label all UV light sources conspicuously with the following warning (or equivalent): "Warning – this device produces potentially harmful UV light. Protect eyes and skin from exposure."

6.1.72. Ensure that the UV light source is shielded.

6.1.73. Ensure that appropriate PPE is worn and is sufficient to protect the eyes and skin. PPE should include at least UV resistant face shield, gloves, and lab coat.

6.1.74. Shielding the equipment or the work area may be warranted.

7. ELECTRICAL SAFETY

7.1. Report defects/faults to your supervisor.

7.2. **All electrical apparatus must be properly grounded.**

7.3. **Never remove the ground pin of a 3-pronged plug.**

7.4. Inspect electrical cords regularly and have frayed or damaged cords replaced.

7.5. "Piggy-backing" of extension cords is prohibited.

7.6. Never use a power bar beneath workbenches where chemicals are handled.

7.7. **DO NOT** use electric wires as supports and never pull on live wires.

7.8. Ensure that all wires are dry before plugging into circuits.

7.9. **Electrical devices (unless certified explosion-proof) should not be connected outside of the hood** to avoid sparks which may ignite a flammable or explosive chemical.

7.10. Use of Ground Fault Interrupter Circuits (GFCI)is preferable in receptacles located near sinks.

7.11. Circuit breaker panels within laboratories must be easily accessible and clearly marked. Familiarize yourself with their location.

7.12. Only qualified and trained people should repair or modify electrical or electronic equipment.

7.13. Any electrical equipment purchased, regardless of voltage, must be approved as

indicated by the presence of a field approval mark from the Canadian Standards Association (CSA), Electrical Safety Authority (ESA), or an equivalent field approval mark acceptable under the Electrical Safety Code i.e. BC Electrical Code Regulation, International Approval Services (IAS) or Intertek Testing Services. The cost of the BC Safety Authority field approvals and modifications, if required, is the responsibility of the acquiring department.

Static Electricity and Sparks

7.14. Static electricity and sparks may cause a fire under the right circumstances. Always be conscious of the potential for generating sparks.

7.15. Electrical equipment must have spark protection in areas where there is a danger of fire or explosion.

7.16. Some protection from static electricity and sparks is obtained by proper grounding and bonding of containers and equipment.

7.17. A dry atmosphere promotes the formation of electrical charges.

7.18. Common sources of sparks and static electricity are:

7.18.1. decanting of organic liquids from one metal container to another,

7.18.2. plastic aprons,

7.18.3. metal clamps, nipples or wires used with non-conducting hoses,

7.18.4. gases released quickly from cylinders under high pressure,

7.18.5. switches and thermostats, and

7.18.6. electrical contacts (e.g. light switches and thermocouples, refrigerators) may produce sparks.

8. STANDARD OPERATION FOR AUTOCLAVES IN S365

Risks associated with autoclaves

8.1. Autoclaves are sterilizers using high pressure and high temperature steam. The potential safety risks for the operators are:

8.1.1. Heat burns -from hot materials and autoclave chamber walls and door.

8.1.2. Steam burns -from residual steam coming out from autoclave and materials on completion of cycle.

8.1.3. Hot fluid scalds- from boiling liquids and spillage in autoclave.

8.1.4. Hand and arm injuries when closing the door.

8.1.5. Body injury if there is an explosion.

8.2. Equipment to protect against scalds and burns:

8.2.1. Heat-insulating gloves that provide complete coverage of hands and forearms.

8.2.2. Closed-toed footwear.

Operator instructions training

8.3. All operators must have successfully completed an authorized training session on the safe operating procedures of this autoclaves. This requirement applies to both new and experienced personnel. A list of authorized users will be kept with the cycle records.

Before autoclaving the following must be completed

8.4. Before turning the autoclave on:

8.4.1. Drain the blue generator by:

a) opening the tap(labeled #1) at the bottom of the generator,

b) opening the tap (labeled #2) on the side of the autoclave facing the wall to the lab, and

c) allow the water to drain for 5 minutes.

8.5. Close the taps that were opened to drain the generator.

8.6. Turn on the power supply switch on the wall beside the autoclave (labeled power switch).

8.7. Turn on the water supply lever labeled water supply. (it is on when it is parallel with the water supply pipe and off when it is perpendicular to the pipe)

8.8. Allow the pressure in the jacket to come up to 15 pounds before starting the autoclave. (about 30 minutes)

Material Preparation

8.9. Ensure that the material is able to be autoclaved. Samples containing solvents or substances that may emit toxic fumes should not be autoclaved.

8.10. Glassware must be inspected for cracks prior to autoclaving.

8.11. Prepare and package material suitably:

8.11.1. Loose dry materials must be wrapped or bagged in steam-penetrable paper or loosely covered with aluminum foil. Wrapping too tightly will impede steam penetration, decreasing efficiency of the process.

8.11.2. All containers must be covered by a loosened lid or steam-penetrable bung.

8.11.3. Containers of liquid must be a maximum of 2/3 full, with lids loosened.

8.11.4. Glassware must be heat-resistant borosilicate.

8.11.5. Plastics must be heat-resistant e.g.: polycarbonate (PC), PTFE ("Teflon") and most polypropylene (PP) items.

8.11.6. Items or containers must be tagged with autoclave tape to verify sterilization.

8.11.7. Loosen all lids to prevent pressure buildup.

8.11.8. Add water to containers as appropriate.

8.12. Place items in containers to secure and contain spills:

8.12.1. items should be placed in a stainless steel or autoclavable plastic container for their stability and ease of handling,

8.12.2. place containers of liquid, bags of agar plates, or other materials that may boil over or leak, into a secondary pan in the autoclave,

8.12.3. the pan must be large enough to contain a total spill of the contents,

8.12.4. bags must not be tightly sealed as steam cannot penetrate, and

8.12.5. remove all labels from glassware prior to autoclaving.

8.13. Biohazard materials must be labeled as such and secured in containment vessels or autoclavable bags and processed as soon as possible according to requirements for the handling of infectious or biohazard materials.

Loading Autoclave

8.14. Wear heat-insulating gloves, and closed toed shoes.

8.15. Place material in autoclave. Do not mix incompatible materials.

8.16. Do not overload; leave sufficient room for steam circulation. If necessary, place the container on its side to maximize steam penetration and avoid entrapment of air.

8.17. Close and latch door firmly by raising the lid gently till it clicks into place.

8.18. Do Not Let it Slam at the top as it will break the switch located there.

Operating Autoclave

8.19. Choose appropriate cycle (e.g. liquid, dry unwrapped or wrapped etc.) for the material.

8.20. Set appropriate temperature for the cycle (if necessary, usually all loads are processed at 121° C.

8.21. Press the start button.

8.22. Do not attempt to open the door while autoclave is operating.

8.23. The manuals for operation of the autoclave are located in the cupboard adjacent to small autoclave and under behind door panel of large autoclave.

Unloading Autoclave

8.24. Wear heat-insulating gloves and closed toed shoes.

8.25. Ensure the load is complete.

8.26. Wear gloves and stand back from the door as a precaution, carefully crack door open no more than 1 inch (2.5 cm) to release residual steam and allow pressure within liquids and containers to normalize.

8.27. Allow sterilized material to stand for 10 minutes in the chamber. This will allow steam to clear and trapped air to escape from hot liquids, reducing risk to operator.

8.28. Do not agitate containers of super-heated liquids or remove caps before unloading.

8.29. After removal from the autoclave, place liquid agar in the water bath in the media area that should be turned on before starting the load. This will allow the media to cool to a temp ideal for pouring.

9. CONTINGENCY PLAN

Equipment malfunction

9.1. If the autoclave does not operate exactly as expected, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.

9.2. Record the problem in the autoclave log book. Contact May or Shannon to report the problem.

9.3. Repair of autoclaves shall be performed by qualified persons only.

10. INCIDENT RESPONSE

10.1. All incidents are to be reported to May or Shannon.

10.2. If any injury occurs seek first aid or, if necessary, seek medical assistance by dialing security at 5033.

10.3. If clothing is soaked in hot water/steam, remove clothing and cool the injured part in cool water.

10.4. Place a notice on the autoclave indicating that it is not to be used until the cause of the incident is determined, procedures enacted to prevent future incidents, and the autoclave is deemed safe for operation.

Spill clean-up

10.5. Spills may occur from a boil over or breakage of containers.

10.6. No operation of the autoclave is allowed until the spill is cleaned up.

10.7. The operator is responsible for cleanup of spills. Contain the spilled material using paper towel, to absorb or contain the spill. Wait until the autoclave and materials have cooled to room temperature. Review the MSDS (found on-line using the MSDS online icon on the desk top, if appropriate, to determine the protective equipment, spill cleanup and disposal protocols that are necessary. Clean the equipment and work area in order to collect and remove all spilled materials. Dispose of the waste following the protocol appropriate for the material. If materials have been intermingled, follow the cleanup and disposal protocol for the most hazardous component of the mixture.

10.8. Cracked glassware must be disposed of properly in the "Broken Glass" disposal pail.

10.9. Report it to Carolynne.

11. ETHIDIUM BROMIDE CLEAN UP PROCEDURE

11.1. Ethidium Bromide is a very common fluorescent intercalating agent used for visualization of nucleic acids. It is sometimes the cause of health and safety concerns for workers charged with using it during the course of their work. Its toxicological properties are not fully determined. There is no evidence supporting or refuting carcinogenicity in humans. Ethidium bromide has been used as an anti-tumorigenic agent in rats and is considered to be non-carcinogenic in rats and mice. It has been found to be mutagenic and genotoxic in various short term in vitro tests such as the Ames test. The precautionary principle suggests that Ethidium bromide be treated as a carcinogen despite the lack of conclusive evidence. Ethidium bromide is toxic with a fairly low LD50 of 50-110mg/kg.

Clean up Procedure

11.2. PPE to be worn – nitrile gloves, lab coat, closed toe shoes, UV filtering eyewear (for use with UV light).

11.3. Using ethanol, wipe down surfaces with a rag or disposable cloth.

11.4. To test for removal, use a UV lamp (black light) to find ethidium bromide that has not been removed. The ethidium bromide will give off a characteristic orange colour. Re-clean areas that were initially missed.

11.5. Dispose all wipes and gloves used for removal as chemical waste.

11.6. Wash hands and any other area that may have contacted ethidium bromide. Wash lab coat after completion of cleanup.

12. CLEANING & MAINTENANCE

Centrifuges

12.1.1. Maintenance and repairs can only be performed by trained, qualified personnel.

Gas Chromatographs

12.1.2. The oven must be cooled before maintenance is performed.

12.1.3. The power cord must be disconnected prior to performing maintenance.

12.1.4. Only qualified, trained personnel can perform maintenance and repairs.

High Performance Liquid Chromatographs

12.1.5. The power cord must be disconnected when performing routine maintenance.

12.1.6. Repairs can only be performed by trained, qualified personnel.

Autoclave

12.1.7. No person shall operate the autoclave unless the autoclave is in good repair.

12.1.8. Users are not to make repairs.

12.1.9. Report possible malfunctions to May or Shannon.

13. REFERENCES

National Toxicology Program (August 15 2005), Executive Summary Ethidium Bromide: Evidence for Possible Carcinogenic Activity, http://ntp.niehs.nih.gov/?objectid=6F5F63F6-F1F6-975E-79965F7EE68AE7C0. Viewed October 2009

Hengen P. N.,1994, Methods and Reagents: Disposal of Ethidium Bromide, Trends in Biochemical Sciences 19 (6): 257-258. doi:10.1016/0968-0004(94)90152-X

14. RECORDS/VERIFICATION OF UNDERSTANDING

14.1. Records:

14.1.1. List of authorized users for the S365 autoclaves kept with the cycle records

14.1.2. Lab Risk Assessment and Control Records

14.1.3. Student Competency Checklists

15 Verification of Understanding:

15.1 A training master log will be maintained by the TRU Biology Department and chairperson of that department

16 SUMMARY OF CHANGES

Revision #	Date	Change (include section #)	Issued By
1	2014.03.10	NEW	OHS Officer
2	09/29/2014	Revisions	OHS Officer