Turtle blood sample processing

Standard Operating Procedure (SOP)

Type of SOP: ☒ Process/Equipment ☒ Hazardous Chemical

All personnel who are subject to these SOP requirements must review a completed SOP and sign the associated training record. The most current version of the SOP is in electronic form on the University's Share Drive. Follow the link below.

S:\University Info\General Information\Emergency Information\Standard Operating Procedures

In addition to the electronic copies, hard copies of the SOPs can be found inside the laboratory, if the SOP pertains to something within a laboratory.

Date SOP Written: 7/27/22	**Approval Date:** 1-11-2024
SOP Prepared By: Leigh Anne Harden and Mark Poch	
SOP Reviewed and Approved by CHO (signature): Rose Rakers	
☒ **Department: Biology**	☐ **Campus Wide** ☒ **Lisle** ☐ **Mesa**
Principal Investigator (PI): Leigh Anne Harden	**Phone:** 630-829-6562
Principal Investigator (PI): Mark Poch	**Phone:**
Chemical Hygiene Officer (CHO): Dr. Rose Rakers	**Phone:** 630-829-6571
Emergency Contact: Campus Safety Dispatch	**Phone:** 630-829-6122
Location(s) covered by SOP: Building and Room #(s): BK 104/105/150	

1. **HAZARD OVERVIEW** (What are the hazards?) (i.e., hazards associated are chemicals, fire/explosion, electrical, ergonomic)

REQUIRED – Add a brief description of the process involving hazardous chemicals or equipment covered by this SOP.

1. Work with 8 blood samples at a time maximum.
2. Thaw whole blood samples for 30 minutes at room temperature.
3. Vortex samples immediately before an aliquot of blood is taken out, then placed on ice for temporary storage until they are returned to the freezer.
4. Pipet 0.100 mL of blood sample into a 15mL metal free centrifuge tube. Use a new pipet tip for each sample.
5. Pipet 0.300 mL of concentrated Nitric Acid (HNO_3) directly into the centrifuge tube containing sample, being careful not to splash. Never put the pipet tip directly into the tube or touch the walls of the tub. If acid remains on the pipet tip or you accidentally bump it into the walls of the tube, replace it with a new one and

dispose of the used tip into a labeled container for nitric acid waste. Nitric Acid volumes are 3:1 acid to blood sample.

6. Centrifuge the samples to bring all material to the bottom of the tube (2000 rpm for 1 minute)
7. Sonicate samples for 1 hour in water bath sonicator. Water should be deionized water filled to line. Centrifuge tubes should be placed in a durable plastic tube stand and that stand is placed into the water bath. Close the lid. Turn on sonicator and select 22°C water temperature and select sonication setting only for 60 min.
8. After sonication, vent samples in the fume hood to release pressure by removing the cap to each centrifuge tube for several seconds. Samples can remain at this stage for a couple of days.
9. To prepare to use microwave, place kitchen microwave fully into fume hood to allow for door to open. Plug in microwave to turn on.
10. Place samples in a durable plastic tube rack and loosen caps to allow for some gas to escape while heating.
11. Because the minimum volume of aqueous material in a microwave is 50 mL, fill ~8 regular 15 mL centrifuge tubes with 7 mL of water with loosened caps. Place these tubes in another durable plastic tube rack and evenly place onto microwave tray with the samples.
12. Turn on microwave and apply the following procedure:
    - Enter 5-0-0 (for 5 min) on microwave, press power button, then press 2 (for 20% power), please start.
    - Enter 5-0-0 on microwave, press power button, then press 3 (for 30% power), press start.
    - Enter 5-0-0 on microwave, press power button, then press 3, press start.
    - Enter 5-0-0 on microwave, press power button, then press 3, press start.
    - Enter 5-0-0 on microwave, press power button, then press 3, press start.
    - Enter 5-0-0 on microwave, press power button, then press 3, press start.
    - Between each digestion, allow samples to cool for 5 min in the fume hood. Handle samples carefully with a glove as they will be hot/boiling. Once samples have cooled for 1 minute, remove each centrifuge cap for several seconds each to release gas into fume hood. If water has evaporated during digestion period from the water tubes, top off water level in each centrifuge tube back up to 7 mL. place all tubes and racks back into microwave.
    - Periodically, the samples should be centrifuged to return the acid condensation to the base of tube. (2000 rpm for 1 minute)
    - 0.100 mL of hydrogen peroxide may be added to the samples that still producing a lot of gas after step 4, but should be microwaved an additional 5-10 minutes
13. Once samples are fully digested, fill with deionized water to the:
    - 6 mL line to make 5% nitric acid. The dilution factor at this point = 60x (300uL / 6000uL = 0.05 or 5%).
    - 15 mL line to make 2% nitric acid. The dilution factor at this point = 150x (300uL / 15,000uL = 0.05 or 5%).
14. Samples are ready to analyze with the flame atomic absorption spectrometer.

2. HAZARDOUS CHEMICAL(S)

REQUIRED – If the SOP is for a process involving a hazardous chemical(s), provide a list of those chemicals and important properties and signs/symptoms of exposure. List any expected by-products produced if this SOP covers a laboratory process.

Hazardous chemical:
Nitric acid (HNO_3, Omni high purity grade)

Potential Health Effects:
Eye: Causes severe eye burns. Direct contact with liquid may cause blindness or permanent eye damage.

Skin: Causes skin burns. May cause deep, penetrating ulcers of the skin. Concentrated nitric acid dyes human skin yellow on contact.
Ingestion: May cause severe and permanent damage to the digestive tract. Causes gastrointestinal tract burns. May cause perforation of the digestive tract. May cause systemic effects.
Inhalation: Effects may be delayed. Causes chemical burns to the respiratory tract. Inhalation may be fatal as a result of spasm, inflammation, edema of the larynx and bronchi, chemical pneumonitis, and pulmonary edema. Aspiration may lead to pulmonary edema. May cause systemic effects. May cause acute pulmonary edema, asphyxia, chemical pneumonitis, and upper airway obstruction caused by edema. Depending on the conditions, the vapor or fumes of nitric acid may be a mixture of nitric acid and various oxides of nitrogen. The composition may vary with temperature, humidity, and contact with other organic materials.
Chronic: Exposure to high concentrations of nitric acid vapor may cause pneumonitis and pulmonary edema which may be fatal. Symptoms may or may not be delayed. Continued exposure to the vapor & mist of nitric acid may result in a chronic bronchitis, & more severe exposure results in a chemical pneumonitis. The vapor & mists of nitric acid may erode the teeth, particularly affecting the canines & incisors.

3. WHAT ACTIVITIES COULD POSE A RISK?

Activities that could pose a health hazard include:

REQUIRED – Bullet any health hazards associated with the process this SOP is detailing.

Direct contact with liquid on skin or eye while handling nitric acid and transferring to centrifuge tubes via pipettes and flasks.
Accidental ingestion or inhalation of nitric acid liquid or vapors while transferring nitric acid.

Activities that could pose a physical hazard include:

REQUIRED – Bullet any physical hazards associated with the process this SOP is detailing.

Handling boiling microwaved water in centrifuge tube without protective gloves.
Physical hazards related to using a microwave, centrifuge, and sonicator.

4. HOW CAN EXPOSURES BE MINIMIZED?

Elimination/Substitution – **REQUIRED** – If there is another chemical or piece of equipment that can be used which is a safer option but that would have a negative effect on the experiment detail why this is not an option.

Not applicable

Engineering Controls – **REQUIRED** – Insert descriptions of lab-specific engineering or ventilation controls used to reduce chemical exposures (i.e., fume hoods, glove boxes, biosafety cabinets, etc.) or specific equipment safety features. Refer to Section 7 and/or 8 of SDS for proper use.

Any transferring of the manufacturer's bottle of nitric acid from acid storage cabinet to the lab will be done in a secondary safety container.
Any transferring of nitric acid from manufacturers bottle to centrifuge tubes and flasks will be done in a fume hood.

Any microwaving of samples will be done in a fume hood behind safety glass of fume hood.

Administrative Controls

The following elements are required:

1. Complete the Hazard Communication and/or Laboratory Safety training prior to working in the laboratory;
2. Complete laboratory-specific safety orientation and training on laboratory-specific safety equipment, procedures, and techniques to be used, including a review of the Chemical Hygiene Plan, prior to receiving unescorted access to the laboratory;
3. Sign off that you read and understand the Chemical Hygiene Plan and what is expected while working in the laboratory;
4. Be familiar with the location and content of any applicable Safety Data Sheets (SDSs) for the chemicals to be used;
5. Implement good laboratory practices, including good workspace hygiene;
6. Inspect all equipment and experimental set-ups prior to use;
7. Follow best practices for the movement, handling, and storage of hazardous chemicals. An appropriate spill clean-up kit should be in the laboratory. Chemical and hazardous waste storage must follow an appropriate segregation scheme and include appropriate labeling. Hazardous chemical waste must be properly labelled, stored in closed containers, in secondary containment, and in a designated location;
8. Do not deviate from the instructions described in this SOP without prior discussion and approval from the PI and CHO; and
9. Notify the PI, CHO and Emergency Preparedness Manager of any accidents, incidents, near-misses, or upset conditions (i.e., unexpected rise or drop in temperature, color or phase change, evolution of gas) involving the process or hazardous chemical(s) described in this SOP.

REQUIRED – Add any additional administrative controls specifically related to the process, procedure, and restrictions, including controls that may be chemical-specific.

All work with concentrated acids must be done in the fume hood, with no other persons working close enough to interfere or come into contact with the acid. A co-worker must be present in the immediate area in case of an emergency, the protective shield on the fume hood drawn down as much as possible, but allowing to work comfortably. Make sure when you are dispensing the acids that the bottle being dispensed in is far enough in the fume hood so that fumes will not come back to the users face (6 inches from the sash is the optimal distance).

Personal Protective Equipment (PPE)

At a minimum, long pants (covered legs) and closed toe/closed heel shoes (covered feet) are required to enter a laboratory or technical area where hazardous chemicals are used or stored. In addition to the minimum attire required upon entering a laboratory, the following PPE is required for all work with hazardous chemicals:

A. Eye Protection
    a. Eye protection must be ANSI Z87.1 compliant.
    b. At a minimum, safety glasses are necessary.
    c. Splash goggles must be substituted for safety glasses in chemistry laboratories, and are required for processes where splashes are foreseeable or when generating aerosols.

B. Body Protection: At a minimum, a chemically-compatible laboratory coat that fully extends to the wrist is necessary.
    a. If a risk of fire exists, a flame-resistant laboratory coat that is NFPA 2112 compliant should be worn.
    b. For chemicals that are corrosive and/or toxic by skin contact/absorption additional protective clothing (i.e., face shield, chemically-resistant apron, disposable sleeves, etc.) are required where splashes or skin contact is foreseeable as per the SDS.
C. Hand Protection: Hand protection is needed for the activities described in this SOP. Define the type of glove to be used based on the following:
    a. Chemical(s) being used;
    b. Anticipated chemical contact;
    c. Manufacturer' permeation/compatibility data; and
    d. Whether a combination of different gloves is needed for any specific procedural step or task.

REQUIRED – Add any descriptions of PPE and hygiene practices used with each process or hazardous chemical(s), including any specialized PPE needed for a procedural step/task.

Butyl rubber gloves are to be used when transferring nitric acid from the manufacturer's bottle to the secondary bottle. Nitrile gloves may be used for pipetting the small aliquots needed for digestion.
Hot plate gloves should be used for handling hot centrifuge tubes.

5. ADDITIONAL GUIDANCE

Spill and Emergency Procedures

Follow the guidance for chemical spill clean-up from the SDS, unless specialized clean-up procedures are described in detail below.

INSERT – Descriptions of any specialized spill clean-up procedures for the hazardous chemicals used in this SOP. Additional details of lab-specific spill clean-up should be provided.

If you accidentally spilled concentrated acid (e.g., while pouring an acid into the measuring cylinder): Do not panic! Remain calm. If the spill is minor (less than 30mL, "note a 5-inch x 7-inch paper towel will absorb 5 ml of water") and will not pose a health issue; use sodium carbonate to neutralize the acid. Pour the absorbent; first make a circle encompassing the spill then pour the absorbent on top of the spill. Find a plastic/neoprene-disposal bottle and carefully place the soaked absorbent into the bottle, and place a chemical waste label on the container. To minimize contact with the acid during clean-up, use the small broom and dust pan to move the soaked absorbent into the bottle. All of this must be done in the fume hood. If the spill is significant; larger (> 30 mL) or you flipped over an entire bottle of acid, if the spill does not pose a health hazard; inform any other personnel in the lab; then use the spill-containment kit underneath paper towel dispenser. Pour the absorbent around the spill then pour the absorbent all over the spill. If spill is greater than 30 ml, or cleanup takes longer than 15 minutes, document the cleanup activity. **IF THE CONCENTRATED ACID IS RUNNING OUT OF THE FUMEHOOD**, place spill-containment absorbent on the spill and contact campus safety immediately and leave the laboratory making sure you notify any personnel in that lab, but stay nearby to provide information to responders. DO NOT TRY to place the soaked absorbent into the neoprene bottle, as fume evaporating from the concentrated acids pose a serious health hazard. If a fire breaks out because of the acid spill leave the area immediately, activate the fire alarm, and call 911. Stay close to the building to inform emergency personnel of what started the fire and what other chemicals are in the immediate area of the fire.

Disposal and Decontamination Procedures

Hazardous waste must be properly labeled and removed from your laboratory within six (6) months of the accumulation start date. Hazardous waste should be brought down to the Chemical Stockroom. Speak with the CHO prior to bringing the waste.

REQUIRED – Add descriptions of laboratory-specific information on the waste streams generated, storage location, and any special handling/storage requirements.

2% Nitric Acid aqueous solutions in centrifuge tubes will be disposed of in an appropriately labeled waste container. This waste container will be stored in a cabinet labeled Nitric Acid Solutions. Waste containers/receptacles should be handled with nitrile gloves and splash goggles.

REQUIRED – Add descriptions of decontamination procedures for equipment, glassware, and controlled areas (i.e., glove boxes, fume hood).

All glassware will be rinsed thoroughly with deionized water before use. Sonicator water bath will be emptied after samples sonicated. Fume hood and bench space will be wiped down each day. Microwave will be cleaned after the samples are digested.

Upon completion of work with hazardous chemicals and/or decontamination of equipment, remove gloves and/or PPE to wash hands and arms with soap and water. Additionally, upon leaving a designed hazardous chemical work area remove all PPE worn and wash hands, forearms, face, and neck as needed. Contaminated clothing or PPE should not be worn outside the lab. Grossly contaminated clothing/PPE and disposable gloves must not be reused.

Shipping and Transportation

Follow the Shipping Hazardous Materials policy found at **S:\University Info\General Information\Emergency Information**.

Fires

INSERT – Add descriptions of what to do in case of a fire as described by the process mentioned in this SOP.

If a fire occurs in your room: • Leave immediately and close the door behind you, but DON'T LOCK IT. If you lock your door, firefighters will have to break in. • Exit from the safest stairwell and leave the building. Once you reach a safe location outside, call 911 and then the University Campus Safety at (630) 829-6122 and provide the dispatcher with your address, floor, and room number. • Never go upstairs because smoke and heat rise. • Do not use elevators. They are to be used by the fire department only.

Exposure Requiring the Use of Emergency Shower and/or Eyewash/Drench Hose

- Have someone call 911 (report the building name, street address (located near the door to the room on the Emergency Procedures sheet) and room number.
- Contact Campus Safety at 630-829-6122 to report the incident and let them know you called 911.
- Have someone obtain the SDS for the material and provide it to the first responders upon arrival.

- Assist the affected individual to position their head over the eyewash/drench hose located in the laboratory and activate it if the eyes or face are affected. If the exposure is on the body assist the affected individual to the emergency shower in the hallway and activate it. The activation of either the eyewash or shower located in the hallway will trigger an alarm notifying Campus Safety. Ensure your own safety before helping others. Only help if it is safe for you to do so.
- Instruct the affected individual to open their eyes and roll them around while the water is flowing or to stand under the shower with the affected area being covered in water.
- Flush the affected area for 15 minutes with water.
- Notify the Emergency Preparedness Manager as soon as possible and complete the Accident/Incident Form.

6. TRAINING

To teach and learn inside a laboratory, certain training must take place. All individuals must take a Laboratory Safety online course. If your laboratory involves chemicals for chemistry or biology, individuals must also take the Hazard Communication online course. These two online courses are set up with the Emergency Preparedness Manager.

Refresher training for the Hazard Communication course will be taken if the individual completed the full three-part course within six (6) months. If it has been close to or over one (1) year, the full three-part course will need to be repeated. The Laboratory Safety course will be repeated if the individual completed the course over one (1) year prior.

In addition to the online courses, students are required to complete laboratory-specific training to be able to stay in the laboratory.

7. SOURCES AND ADDITIONAL RESOURCES

List all sources and additional resources that contributed to the creation of this SOP.

https://fscimage.fishersci.com/msds/16550.htm
Blood Digestion Protocol from Environmental & Occupational Health Sciences Institute of Rutgers University

All personnel shall read and fully adhere to and acknowledge the contents, requirements, and responsibilities outlined in this SOP.

☐ I have read and acknowledge the contents, requirements, and responsibilities outlined in this SOP.

Print Name:	Signature:
BenU ID:	Date: