Safe Work Practices
Centrifuge Use

Scope and Purpose

This Safe Work Practice document covers some of the hazards and provides some general safety considerations for operating a centrifuge. The purpose of this document is to assist supervisors in the development of their lab-specific Standard Operating Procedure (SOP) for using and maintaining their centrifuge. This document does not take the place nor does it fulfill the role of a detailed work-specific, safety focused SOP. Before any personnel uses a centrifuge they must have received in-house training and have reviewed their lab's specific centrifuge SOPs.

As with the operation of any equipment, the manufacturer's instructions on safe use and required maintenance should be followed.

Introduction

Centrifuges are a commonly used piece of laboratory equipment. Centrifuges spin at high speeds, producing centrifugal force, to separate substances with different particle sizes or densities.

In general, centrifuges are classified as either a floor standing or bench top model. Floor standing models can reach higher speeds and have greater capacity. Floor standing models include ultracentrifuges, super speed centrifuges and low speed centrifuges. Bench top centrifuges are available in different sizes. Microcentrifuges are used for spinning low volume tubes. There are also multipurpose bench top centrifuges which allow for a broad range of rotors and can be adapted to spin a wide variety of tubes and bottles.

There are a variety of rotors that can be used in centrifuges including fixed angle, swinging bucket and vertical rotors. Use only those rotors designed for use in your particular centrifuge.

All centrifuges, including microcentrifuges, can be hazardous if not used or maintained properly. Before any work with centrifuges can occur in your lab a Local Risk Assessment (LRA) needs to be completed and a work-specific SOP created.

Responsibilities

Principal Investigator/Supervisor:

- Perform a LRA for the proposed work
- Create a written SOP for the safe operation of the centrifuge (and rotors)
- Ensure all users are trained in the safe operation and maintenance procedures of the centrifuge and rotors to be used
- Document all training
- Validate the competence of users while operating the machinery and provide refresher training when appropriate
- Supervise centrifuge use when/if necessary
- Ensure that copies of the user instructions for the equipment are readily available
- Ensure that all routine maintenance and repairs are conducted as per instructions outlined in SOP

Users:

- Complete a training session on centrifuge (and rotor) use and maintenance
- Read and follow all provided instructions (SOPs) for the safe use and maintenance of the equipment
- Wear required PPE
- Operate and maintain all centrifuges and rotors according to the outlined safety procedure and lab protocol (SOP)
- Report any cause for concern or issues when operating the equipment to the responsible lab operator or supervisor
- Report any accidents (injuries, spills) or near-miss incidents to your supervisor immediately

Local Risk Assessment (LRA)

A risk assessment for the use of a centrifuge in your lab must be performed. This assessment should be prepared by an experienced person who is competent and knowledgeable in the operation of centrifuges and potential risks and hazards. This assessment must identify the hazards including the biohazards posed by the material being worked with and who might be harmed. The LRA must also assess the likelihood of injury and its consequences and outline measures to be used to reduce the risk of injury. The risk assessment can then be used to help create your lab's work-specific, safety focused SOP.

For more details on LRAs see Canadian Biosafety Handbook (CBH) 4.4.1 and Canadian Biosafety Guideline – Local Risk Assessment: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/guidance/canadian-biosafety-guidelines.html

The Canadian Biosafety Handbook (CBH): https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html

Standard Operating Procedure (SOP)

A Standard Operating Procedure is a detailed step-by-step procedural document on the safe operation of the particular centrifuge used by your lab. It should contain precise, practical instructions on how to use the device and all the safety measures that should be followed including PPE requirements. It should also include maintenance and documentation requirements, waste and decontamination instructions, emergency procedures and accident reporting.

Remember to check all manufacturer recommendations on safe use and maintenance and incorporate them into your SOP.

The SOP should be compiled by an experienced centrifuge user and updated if there are any changes in the equipment or procedures or as necessary. Principal Investigators are responsible for reviewing and approving all SOPs relevant to their laboratory operations. It must be reviewed, understood and followed by all users. The SOP should be included in your lab-specific biosafety manual and be available for review by all users.

You may decide to post the SOP or portions of the document near the centrifuge.

You should have a logbook tracking the usage of the centrifuge. This will assist the lab in determining maintenance schedules, ascertaining when rotors should be retired and keeping records of decontamination, problems and other observations.

For more information on SOPs see Canadian Biosafety Handbook (CBH) 5.3.5.

Personnel Training and Clearance

Personnel must know and understand the potential hazards of their work, and follow all operational practices and procedures. The fundamentals of safe biological work are discussed in the Laboratory Biosafety course (EHS601). Supervisors must also ensure that all personnel, have successfully completed an in-house training session on their lab's SOPs including equipment use, waste procedures and emergency response. Personnel must show understanding and competence in those procedures prior to working in the lab. This requirement is applicable to both new and experienced personnel. All in-house training must be documented, dated and signed by both the trainee and trainer, and available to view upon request by EHS personnel or external regulators. Documentation of all in-house training should be kept by the supervisor for a minimum of 5 years after the personnel has left the lab.

A review of all centrifuge safety measures and emergency response procedures should be part of your annual emergency response review with lab personnel (Canadian Biosafety Standard (CBS) 4.3.10).

The Canadian Biosafety Standard (CBS): https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

General Safety

Personal Protective Equipment (PPE)

The PPE to be worn when working in any lab should be in accordance with the highest risk or possible hazard for the equipment, biological agents, material or chemical used in the procedure. The supervisor should advise what PPE is required based on a LRA to identify potential risks in any lab procedure. PPE must be worn at all times when working with biological agents. Check the Safety Data Sheet(s) (SDSs) for the chemicals that will be used in the procedure, to ascertain if any additional PPE is required.

Centrifuge usage may require specific and additional PPE which must be detailed in your SOP (based on your LRA).

- Lab coats must be long sleeved and knee length
- Long pants or skirt (the entirety of the legs must be covered)
- Shoes are to be closed toe and heel, low heeled (or no heeled) and have non slip soles (CBS 4.6.3)
- Gloves (CBS 4.4.4). Specific glove types may have to be specified for different procedures (based on your LRA). Some examples include nitrile/latex laboratory gloves for handling specimens, and insulated utility gloves for handling freezing materials. Ensure gloves are compatible with possible hazards
- Safety goggles or face shield if there is a possibility of ocular splash, or flying debris (CBS 4.4.2)
- Personnel to remove PPE carefully to minimize possible contamination of their skin, hair or clothing when leaving the containment zone (lab) (CBS 4.5.14)
- Potentially contaminated clothing articles and PPE should be decontaminated prior to washing (CBS 4.8.5, 4.8.6): https://ehs.utoronto.ca/wp-content/uploads/2015/10/Lab-Coat-Washing-Guidelines.pdf

Additional information and resources on PPE are provided below:

General Laboratory PPE Assessment tool: https://ehs.utoronto.ca/wp-content/uploads/2016/06/Laboratory-PPE-Assessment-Tool.pd-Updated.pdf

General information on PPE: https://ehs.utoronto.ca/resources/personal-protective-equipment-ppe/

Protective Glove Standard: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Hand-Protection-Gloves.pdf

Protective Eye and Face wear Standard: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Eye-Protection-Standard.pdf

Respiratory Protection Program: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Respiratory-Protection-Program.pdf

Containment Level (CL) Requirements

The laboratory space in which you handle biological material must correspond to the highest Risk Group (RG) of that material. For example, if working with Risk Group 2 (RG2) material, the lab space must be commissioned as a Containment Level 2 (CL2) lab and attached to your permit prior to beginning work. Contact the Biosafety Office (ehs.biosafety@utoronto.ca) if you have any questions.

If working with aerosolizable RG2 agents, it is recommended that the centrifuge be located within the laboratory where the bioagents are being handled and that the room has Inward Directional Airflow (IDA). If the centrifuge is located in another lab, then methods for the safe transportation of the bioagents must be outlined in your SOP.

Risks and Hazards

Due to the high speeds at which centrifuges operate there is a potential for users to be harmed. Mechanical failure of the rotors can result in injury and sample container breakage can release aerosols of harmful material. When centrifuging hazardous materials use tightly capped tubes and/or sealed safety cups or rotors that can be loaded and unloaded in a Biological Safety Cabinet (BSC) or fume hood depending on the hazard. Below are listed some of the hazards associated with centrifugation and suggestions on risk reduction. It is very important that your LRA captures the hazards that are present in your proposed work procedures. Risk reduction precautions and techniques need to be explored and incorporated into your SOPs.

Preventive maintenance is a key component to reducing the risks of centrifuging. Your centrifuge should be inspected regularly to ensure that all components are functioning correctly and are not in need of repair or replacement. Your lab should adhere to a maintenance schedule, which includes the cleaning and disinfecting of the centrifuge interior and rotors and the cleaning and lubrication of any O-rings. Maintain a logbook to track usage and retire any rotors after the manufacturer's recommended life span. Reference the centrifuge operating manual or contact the manufacturer for guidance.

Mechanical Hazards

Suggestions for the safe operation of a centrifuge include but are not limited to:

- Always install the centrifuge according to the manufacturer specifications
- Avoid locating the instrument near areas with flammable/combustible reagents or fluids, or where vibration may cause items to fall off nearby shelves

- The centrifuge should be securely anchored by strong suction cups (benchtop models), wheel brakes (floor models) etc. Excessive movement of the instrument can damage components and injure users
- Use only rotors specifically designed for your instrument. For ultra centrifuges, the classification decal on the centrifuge and rotor should match
- Record the purchase date of each rotor, along with manufacturing date and serial number
- Follow manufacturer guidelines regarding when to derate (permanently lower the speed) and when to retire centrifuge rotors. Keep a logbook to record usage of rotors so that hours of usage can be calculated and used to determine when a rotor should be retired
- Inspect the rotor for signs of corrosion or cracking before using. If the rotor is defective, tag it and remove it from use, and inform the lab supervisor of the problem
- Ensure the centrifuge chamber (bowl) and drive shaft are free of scratches, burrs or other damage
- Ensure the rotor is properly attached and seated on the drive shaft
- Check that the safety cups/buckets are properly seated and able to move freely. Swinging bucket rotors are designed to be used with all buckets present, even if some of them are empty. Make sure bucket pairs (the buckets opposite each other) are the same type
- Inspect the inter-lock system to ensure the cover cannot be opened while the rotor is spinning
- Never operate the rotor without the lid or cover closed and locked in place. If the lid cannot be locked, the machine must be removed from service
- Ensure that the rotor lid is on securely
- Never operate the rotor unless it is symmetrically loaded and balanced. Care is required to achieve this
- To balance a load, fill the sample tubes as evenly as possible and place tubes of equal mass opposite each other when loading. If necessary, use sample tubes filled with water to obtain a balanced load. Opposing loads must balance within a certain tolerance as per manufacturer's instructions. The use of a weight scale to ensure this is recommended
- Always use sample tubes or bottles designed for the particular rotor being used
- Inspect all tubes for any damage before use. Sample tubes can become damaged through wear and tear. Discard any tubes that show discolouration, crazing or scratches. Ensure the tube's lid closes properly
- Never exceed the maximum recommended speed of the rotor
- Personnel should remain with the centrifuge until it reaches full speed. Stop the centrifuge immediately if an unusual condition (noise or vibration) begins. Push the centrifuge's stop button and move away from the instrument. When the centrifuge comes to a complete stop, investigate the cause and perform needed corrections before resuming run
- Never touch a rotor that is still moving
- Ensure that the power cord for the centrifuge is accessible at all times. Pull out the power cord at the instrument or electrical outlet, or disconnect the electrical power supply in an emergency
- Clean and disinfect rotors and sample cavities or cups after each use with non- corrosive solutions

- Any repairs to a centrifuge must be done by qualified, manufacturer authorized individuals. Keep records of any repairs

Exposure Hazards

There is a risk of aerosol generation when a centrifuge is used. If working with potentially infectious RG2 biological agents, then sealed safety cups (or rotors) that are loaded and unloaded in a BSC must be used (CBS 4.6.28). See the Canadian Biosafety Handbook (CBH) 12.1 for more information on centrifuging infectious material.

During centrifugation, aerosols can escape during the high-speed spin process. This may occur if you spin uncapped samples or if a tube leaks or breaks. Aerosol-tight rotors and buckets must be used when centrifuging any material that poses a health hazard (e.g. toxic liquids or pathogenic bioagents). Aerosol-tight rotors and buckets use rubber O-rings to make them air tight. These O-rings must be checked periodically (e.g. once per week/month depending on usage) to see if there is any damage. If they are broken or cracked, they must be replaced before use. Document all equipment checks. Follow the manufacturer's instructions for cleaning and lubrication requirements of all rubber seals.

Only centrifuge tubes equipped with O-rings are aerosol tight (Eppendorf tubes and screw cap tubes are not). Even with the use of tubes equipped with O-rings, a centrifuge that has aerosol-tight capability is required when working with aerosolizable RG2 bioagents as the tubes may crack or break, leading to the release of aerosols.

If working with potentially infectious RG2 agents where aerosol production poses a hazard, see the following document for more information on aerosol risk reduction techniques and safety precautions: https://ehs.utoronto.ca/wp-content/uploads/2020/09/Safe-Work-Practices-Aerosol-Risk-Reduction-RG2-Biological-Agents.pdf

Ultracentrifuges:

- Read and follow all manufacturer instructions to prevent damage or malfunction of the device. Follow manufacturer's advice on required maintenance for centrifuge and rotors (i.e. O-ring cleaning and lubrication, rotor retirement)
- Do not overfill centrifuge tubes. Wipe the outside of tubes with disinfectant after they are filled and sealed
- The centrifuge must be equipped with sealed cups (equipment with O-rings/gaskets) which are loaded and unloaded in the BSC (CBS 4.6.28)
- Check the integrity of the cups, O-rings and gaskets before use. If any of these components are defective, they must be replaced
- A maintenance log must be kept to document O-ring change-outs and periodic inspections of the devices. (CBS 4.6.14) A maintenance schedule for the centrifuge outlining all this and other maintenance must be documented in your permit-specific biosafety manual
- Decontaminate the outside surface of cups and rotors after each use

- It is recommended that the centrifuge be located within the laboratory where the bioagents are being handled and that the room has Inward Directional Airflow (IDA). If the centrifuge is located in another lab, then methods for the safe transportation of the bioagents must be included in your SOP (see below for instructions regarding safe transport)

Bench Top Centrifuges:

- Centrifugation of any aerosolizable biological agents must be conducted in an aerosol-tight centrifuge (centrifuge rotor or bucket is o-ringed) which is loaded and unloaded within a BSC (and prior to removal from the BSC, is appropriately disinfected). See ultracentrifuge instructions above for further details
- Aerosols may be produced in bench top centrifuges if using poorly sealed test tubes or Eppendorf tubes
- Microcentrifuges:
    - If spinning aerosolizable materials in a microcentrifuge without aerosol-tight capability, then the microcentrifuge must be used in a BSC. However, equipment which creates air movement may affect the integrity of the airflow and should not be used within the BSC (CBH, chapter 11)
    - While not recommended, if it is necessary to proceed with the placement of the microcentrifuge in the BSC, ensure that the microfuge causes no or very minimal air flow disturbance, is placed towards the back of the BSC (without blocking the rear grille (CBH 11.4.1)), and have your BSC certifier recertify the BSC while the centrifuge is running to ensure that the integrity of the airflow is not compromised

Operator Error

Many of the reported accidents involving centrifuges are caused by human error. Ensure that your SOP instructions are clear, concise and fully understood by your personnel. It is suggested that experienced personnel are available to answer questions or concerns when new personnel first start using your centrifuge. Demonstrate how any new components such as new rotors should be set up and used. Some of the possible human errors that may cause accidents include:

- Failure to place the lid on the rotor.
- Failure to properly secure the rotor lid.
- Failure to properly secure the rotor to the drive.
- Overloading the rotor's maximum mass.
- Running the swinging bucket rotor with missing buckets.
- Buckets hooked incorrectly and unable to swing freely.
- Improper balancing of centrifuge tubes.
- Utilization of centrifuge tubes that are not rated for the correct speed.

Spill in Centrifuge

All personnel must be familiar with the location and use of your biological spill kit and safety features such as emergency eyewashes and showers. For further information on spill procedures see: https://ehs.utoronto.ca/our-services/biosafety/biological-spills/ and CBH 17.3.3

In the event of a spill or tube breakage (actual or suspected):

- If the centrifuge is in operation, switch it off immediately
- Leave lid closed for at least 30 minutes to allow aerosols to settle, if breakage is discovered after the centrifuge is opened, replace lid immediately and leave closed for the required time
- Affix signage not to open the centrifuge
- Move to BSC if possible (see CBH 17.3.3 for detailed instructions)
- Disinfect the centrifuge, rotors and buckets with an appropriate disinfectant; allow at least 20 to 30 min of contact time. Wipe down all parts including the lid and bowl
- Rinse with water if bleach was used
- Report incident to supervisor

Reporting Injuries

Personnel must immediately inform their supervisor of any accident causing injury. Within 24 hours, the supervisor will have to file a report via the University of Toronto EHS website (https://ehs.utoronto.ca/report-an-incident/). Appropriate medical evaluation, surveillance, and treatment must be sought and provided if needed.

Waste Management

For an overview of waste procedures in bio labs see: https://ehs.utoronto.ca/wp-content/uploads/2019/04/Waste-Information-and-Procedures-for-Bio-Labs_v3.1-09-14-2020.pdf

Ensure that waste is disposed into the correct containers. This is a link to the university's various waste disposal streams: https://ehs.utoronto.ca/wp-content/uploads/2015/10/Bucket-List-Poster.pdf

Information regarding Biological Waste packaging and collection can be found at https://ehs.utoronto.ca/laboratory-hazardous-waste-management-and-disposal-manual/biological-waste-disposal/

St. George campus

To set up a pickup service or if you have any questions on hazardous material disposal/waste, contact Rob Provost, Manager, Environmental Protection Services (EPS) at 416-978-7000 or rob.provost@utoronto.ca . For chemical/biological waste buckets or a waste collection contact EPS directly at 416-946-3473,or hazwaste.ehs@utoronto.ca . EPS website: https://ehs.utoronto.ca/our-services/environmental-protection-services/

UTM and UTSC campuses:

Waste is received at established storage areas at either the Central Stores or Receiving Areas at both UTM and UTSC. If unaware of the proper contact at UTM and/or UTSC contact Rob Provost, Manager, EPS at 416-978-7000 or rob.provost@utoronto.ca .

Resources

Algonquin College, Risk Management, ASET SOP – Centrifuges: https://www.algonquincollege.com/safety-security-services/home/occupational-health-and-safety/biosafety-at-ac/biosafety-training-and-emergencies/aset-standard-operating-procedure-centrifuges/

California State University Fullerton, Environmental Health & Safety, Centrifuge Safety: https://hr.fullerton.edu/riskmanagement/_documents/policiesandguidelines/centrifuge.pdf

Creighton University, Environmental Health & Safety, Centrifuge Safety: https://www.creighton.edu/researchservices/ehs/labsafety/centrifugesafety/

Eastern Washington University, Environmental Health & Safety, Centrifuge and Rotor SOP: https://d3tb2mkdocc4em.cloudfront.net/ehs/wp-content/uploads/sites/116/2017/02/Centrifuge-SOP.pdf

Eppendorf Handling Solutions, Centrifugation, Safe Use of Centrifuges https://handling-solutions.eppendorf.com/sample-handling/centrifugation/safe-use-of-centrifuges/

Lab Manager, The Basics of Centrifuge Operation and Maintenance: https://www.labmanager.com/product-focus/the-basics-of-centrifuge-operation-and-maintenance-1433

McMaster University, Biosafety Office, Standard Operating Procedures, Documents, Forms and Templates, Equipment, Centrifuge Safety in Biohazard Lab: https://biosafety.mcmaster.ca/biosafety_SOPs.htm

National University of Singapore, Yong Loo Lin School of Medicine, Risk Assessment: Laboratory Centrifuge: https://www.yumpu.com/en/document/view/12346244/risk-assessment-laboratory-centrifuge-a-laboratory-centrifuge-is-a-

Oregon State University, Environmental Health & Safety, Safety Instruction, Centrifuge Safety: https://ehs.oregonstate.edu/sites/ehs.oregonstate.edu/files/pdf/si/centrifuge_safety_si080.pdf

Public Health Agency of Canada, Canadian Biosafety Handbook: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/handbook-second-edition.html

Public Health Agency of Canada, Canadian Biosafety Standards: https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html

Selectech, 7 Safety Precautions for Centrifugation: http://selectech.co.za/wp-content/uploads/2016/04/7-Safety-Precautions-for-Centrifugation.pdf

Stanford University, Environmental Health & Safety, Centrifuge Safety: https://ehs.stanford.edu/reference/centrifuge-safety

The University of Sydney, School of Molecular Bioscience, SOP, SMB007 Centrifugation: High Speed Floor Centrifuges: https://www.sydney.edu.au/science/molecular_bioscience/ohs/documents/RAs%202016/SMB007_Risk_Assessment_Centrifugation_High_speed_floor_centrifuges.pdf

University of British Colombia, Risk Management Services, SOP – General Centrifuge Safety: http://riskmanagement.sites.olt.ubc.ca/files/2016/05/UBCV-RMS-OHS-SOP-14-002-Centrifuge-Safety.pdf

University of California San Diego, Environmental Health & Safety, How to Use a Centrifuge Safely: https://blink.ucsd.edu/safety/research-lab/laboratory/centrifuge.html#Requirement-for-researchers

University of Massachusetts Amherst, Environmental Health & Safety Fact Sheet, Centrifuge Safety: https://ehs.umass.edu/sites/default/files/Fact%20Sheet%20Centrifuge%20Safety.pdf

University of Michigan School of Public Health, Bridges Lab Protocols, SOP-Centrifuge Safety: http://bridgeslab.sph.umich.edu/protocols/index.php?title=SOP_-_Centrifuge_Safety&mobileaction=toggle_view_desktop

University of Nebraska Lincoln, Environmental Health & Safety, SOP – Centrifuge Safety: https://ehs.unl.edu/sop/s-centrifugesafety.pdf

University of Toronto, Environmental Health & Safety: https://ehs.utoronto.ca/

University of Toronto, Environmental Protection Services, Laboratory Hazardous Waste Management and Disposal Manual: https://ehs.utoronto.ca/laboratory-hazardous-waste-management-and-disposal-manual/

For all additional safety and contact information, please visit our website www.ehs.utoronto.ca