Centrifuge Safety in Biohazard Laboratories

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# Purpose

<<<< This SOP is left in DOCX format so that you may edit it for your own laboratory>>>

The purpose of this SOP is to lay out the responsibilities, equipment and procedures required for setup, use and maintenance of a centrifuge to be used with biohazardous materials.

# Scope

This SOP applies to all persons prescribing the use of and using a centrifuge.

# Definitions

Different centrifuges manufactured in a variety of configurations for various applications. Each variety of centrifuge will have different rotors for specific purposes. Rotors are not interchangeable between different makes and models of centrifuges, unless specified in the manufacturer’s specifications. Your protocol must advise you which type of centrifuge and rotor is required. A few definitions are below; always check the user manual for the specific operation and safety procedure for your equipment.

**Ultracentrifuge (High Speed)** - a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 1 000 000 g

**Table/Bench top** - Versatile multipurpose centrifuges are the most common type, with an RCF up to about 24,000 g, a variety of volume ranges, and the ability to spin plates. They can accommodate different types of rotors, including fixed angle and swinging bucket.

**Microfuge** - are an indispensable tool found in clinical, research and academic laboratories. They can be used for spinning down DNA, protein, blood or other samples. They are available in sizes varying from tiny personal microcentrifuges which accommodate as few as 2 tubes to refrigerated models which accommodate up to 48 0.5ml tubes.

**Rotor types:**

**Fixed angle** - are the most ubiquitous rotors used in centrifugation. The majority are used for basic pelleting applications (differential separations), either to pellet particles from a suspension and discard the excess debris, or to collect the pellet. The cavities in these rotors range in volume from 0.2 mL to 1 L, with speeds ranging from single digits to 1,000,000 × g (relative centrifugal force, RCF).

**Swinging bucket -** are ideal for separating large-volume samples (up to 12 L) at low speeds. A swinging-bucket rotor system consists of three parts: 1) the rotor body attaches to the centrifuge drive and has four or six arms to support the buckets, 2) the buckets are placed onto the arms of the rotor body, and 3) trunnion pins are used to hold the buckets in place.

**Vertical Rotor** – These are fairly specialized; their most common use is during ultracentrifugation for isopycnic separations, specifically for the banding of DNA in cesium chloride. In this type of separation, the density range of the solution contains the same density as the particle of interest; thus the particles will orient within this portion of the gradient.

**BSC:** Biological Safety Cabinet

# Responsibilities

It is the responsibility of the Supervisor to:

* **Reviewing this SOP on a regular basis. Review is to consider and mitigate the risks of spill, loss of containment and exposure or other harm. Refer to Performing Risk Assessments SOP.**
* Ensure all centrifuges that their workers are directed to use, are in good repair and labelled with appropriate contact information for maintenance and repair.
* Ensure and document training of all users.
* Ensure all centrifuges used for radioactive materials are labelled properly.
* Ensure that the safety procedures,centrifuge use, and integrity check logsare kept.
* Ensure all copies of user manuals including rotor/tube selection guidelines are readily available to all users.
* Ensure that all the regular and periodic maintenance (RMM 406) required is carried out and recorded in the maintenance log.
* Ensure records of repair are kept and available for inspection.

It is the responsibility of the worker to:

* Complete all training required for safe operation prior to first use.
* Operate and maintain all centrifuges in accordance with manufacturer’s instructions.
* Select the appropriate rotors/tubes for the protocol in use.
* Fill out the use and integrity check logs each time the instrument is used.
* Report any damage of centrifuge or rotor to the laboratory supervisor immediately so equipment lock-out/tag-out measures (RMM 306) and repairs can be made.
* Users must wear appropriate laboratory PPE (RMM 320) for the containment level of their laboratory and for the work they are to undertake.

# Materials

* Centrifuge
* Balance
* Balancing tubes
* Balancing fluid
* Safety cup (if RG2)
* Secondary transport container (if RG2)
* Disinfectant effective against the materials in use.

# General Safety Measures

Centrifuges are instruments with strong potential for harming users due to the high speed at which they operate: mechanical failure of the rotor can result in injury, even death; and sample container breakage can cause cuts and generate aerosols leading to exposure to infectious materials. Thus, it is very important to act safely when using and maintaining these instruments.

* The centrifuge must be installed according to the manufacturer specifications.
* Do not locate the instrument near areas containing flammable reagents or combustible fluids, or where vibration will cause items to fall off nearby shelves.
* Request instructions from the manufacturer on safe transportation procedures if the centrifuge must be moved to another location and instructions are not in the operation manual.
* Always use sample tubes or bottles designed for the particular rotor being used.
* Proper selection, use and maintenance of rotor(s) are critical to safe operation. Use only rotors designed for use in the instrument. Lack of care can lead to severe personal injury.
* Inspect the rotor for signs of corrosion or cracking before using. If found, do not use the rotor, and inform your supervisor, or the supervisor responsible for the centrifuge of the problem. Post a ‘DO NOT USE’ sign.



*Sample of corrosion inside buckets[[1]](#footnote-1)*

* Never operate the rotor unless it is symmetrically loaded and balanced.
* 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. Inform your supervisor, or the supervisor responsible for the centrifuge of the problem. Post a ‘DO NOT USE’ sign.
* Never exceed the maximum recommended speed of the rotor.
* Clean and disinfect rotors and sample cavities or cups after each use with non- corrosive solutions.
* Samples must be securely sealed avoid generation of aerosols.
* Dispose of all waste solutions according to RMM 502.
* Do not lean or place items on the instrument while it is operating.
* Do not leave the centrifuge until full operating speed is attained, and the instrument appears to be running normally without vibration.
* Return to the centrifuge before the end of the run to detect any audible indicators of a malfunction.
* If vibration occurs at the full operating speed, stop the run immediately; wait until the rotor stops, and proceed with Cleaning a Biological Spill inside a Centrifuge SOP.

# Integrity Documentation Procedure

Due to the possibility of aerosol generation leading to exposure to infectious materials while using a centrifuge; all BSL2 and 3 labs must complete and document their integrity checks prior to each use. This procedure is recommended for BSL 1 labs. (see appendix A for a sample log sheet).

* Visually check the integrity of the centrifuge safety cups and lids to ensure there is no cracks and/or chips present, check that the o-ring seal is in place and there is no damage or cracks present, and that all parts are functioning as intended. Follow the manufacturers guidelines for minimum working standards for parts and seals.
* To record integrity check; add a column to your existing centrifuge log indicating all sealed parts were inspected and in good working order or refer to Appendix A for an editable sample Centrifuge Integrity Log.
* In the event of damage, do not use, document issue on log, and report to the responsible person.

# Emergency Procedure

* In the event that an incident or accident related to centrifugation occurs proceed with Cleaning a Biological Spill inside a Centrifuge SOP.

# Operational Procedure

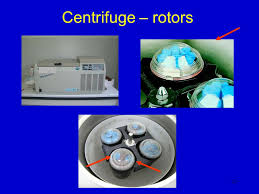
General procedure for centrifuge operation – always follow manufacturer instructions for the specific operation of your model.

1. Determine the appropriate rotor type for your application (swinging or fixed angle), and confirm the tubes you intend to use are compatible. Using the wrong tube[[2]](#footnote-2) may cause damage to the rotor, a spill or the loss of your sample. Rubber adapter sleeves are available for certain tubes.
2. If the samples will be centrifuged at low temperatures, pre-chill the rotor and ensure the centrifuge chamber temperature is set appropriately.
3. Wear the appropriate PPE for the materials you are handling (lab coat, gloves, eye protection etc.).
4. Inspect primary sample tubes for small cracks.
5. Balance tubes according to weight if using a digital scale or use a beam balance. If necessary, use clean tubes for balancing. Never fill centrifuge tubes beyond their maximum rated volume.
6. Place tubes in a secondary transport container for transport to the centrifuge. If required, use a cart.
7. Symmetrically distribute balanced buckets or tubes. Always operate the centrifuge with all buckets in place, even if two opposing buckets are empty.
8. Switch on and follow the manufacturer's instructions to set the centrifugation conditions.
9. Close and lock the lid.
10. Start the centrifuge cycle.
11. Do not leave the centrifuge until full operating speed is attained, and the instrument appears to be running normally without vibration.
12. If vibration occurs at the full operating speed, stop the run immediately; wait until the rotor stops, and proceed with Cleaning a Biological Spill inside a Centrifuge SOP.
13. If centrifuge cycle is successful, wait until the centrifuge comes to a complete stop prior to opening up the unit.
14. Open the centrifuge carefully.
15. Remove the sealed buckets (or tubes) slowly and carefully to prevent re-suspension of the sediments; always check for damage to your tubes.
16. Sediments and supernatants should be visible after centrifugation.
17. When the centrifuge is under refrigeration, leave the top closed to avoid condensation.

# Additional Procedures for use with RG2 and RG2+ Materials

The centrifuge must have either:

* O-ring sealed safety buckets or cups that can be removed from the centrifuge and placed inside a BSC for the removal of individual centrifuge tubes. The sealed buckets or cups protect operators from infectious aerosols in case of tube damage during centrifugation. OR
* a sealed rotor; if safety buckets or cups are not available, the rotor must have a sealing lid to contain aerosols from escaping in the event of an accident during operation



1. Inspect integrity of centrifuge safety cups, lids, rotors, and o-rings and complete integrity check log.
2. Follow Operational Protocols (above) sections 1 – 5.
3. RG2, RG2+ samples must be prepared in the BSC. Remember to always spray tubes, secondary containment device, buckets/safety cups out of the BSC.
4. Transport of sample tubes to the centrifuge or balance must be carried out according to SOP – Transportation and Movement of Biohazards.
5. When balanced tubes, buckets are loaded in the centrifuge, follow Operational Procedures (above) sections 7 – 11.
6. Close and lock the lid.
7. Start the centrifuge cycle.
8. If any excessive vibration, malfunction or breakage is detected/suspected, follow “Cleaning Biological Spills Inside a Centrifuge” SOP
9. If centrifuge cycle is successful, wait until the centrifuge comes to a complete stop prior to opening up the unit.
10. Open the centrifuge carefully.
11. Remove the sealed buckets, rotor, or tubes slowly and carefully to prevent re-suspension of the sediments.
12. Place the buckets, rotor, or samples inside the BSC. In the BSC, carefully open the buckets or rotor; check for tube damage before removing tubes from the buckets.
13. Once samples have been removed from the buckets, clean the interior and exterior of buckets and lids prior to removing them from the Biosafety cabinet (ensure the inside is cleaned and disinfected completely). Spray the buckets out of the cabinet.



*After centrifugation, Open sealed buckets/cups inside BSC[[3]](#footnote-3)*

# References

[Canadian Biosafety Standards (CBS) 2nd Edition](http://canadianbiosafetystandards.collaboration.gc.ca/cbs-ncb/index-eng.php)

[McMaster University, Risk Management Manual: RMM 600 Biosafety](http://www.workingatmcmaster.ca/rmm/)

Pathogen Safety Data Sheets [and](http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php) Risk Assessment

[Biosafety SOP-005 Transportation and Movement of Biohazards](https://biosafety.mcmaster.ca/biosafety_SOPs.htm)

[Biosafety SOP-00X Cleaning a Biological Spill Inside a Centrifuge](https://biosafety.mcmaster.ca/biosafety_SOPs.htm)

Risk Management Manual 102 OHSA

[PHAC Spills Protocols](https://biosafety.mcmaster.ca/documents/doc105_phac_spill_procedures.pdf) (amended)

[Risk Management Manual 502](http://www.workingatmcmaster.ca/rmm/) Waste Management

*Adapted from University of British Columbia, Standford University*

## Appendix A – Sample of Centrifuge Integrity Log

Instructions: Check the integrity of the centrifuge safety cups and lids by visually checking all pieces for cracks and/or chips. Check that the o-ring seal is in place and there is no damage or cracks present. In the event of damage, do not use and report to the responsible person.

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1. Taken from Laboratory Centrifuges, [http://www.centrifuges.co.uk/used.htm#](http://www.centrifuges.co.uk/used.htm) [↑](#footnote-ref-1)
2. Tubes include disposable or reusable/autoclavable, however either must be rated for use with the centrifuge, the maximum speed and the reagents used. [↑](#footnote-ref-2)
3. Taken from Medical University of South Carolina, Biosafety department, www.musc.edu [↑](#footnote-ref-3)