

Introduction

The purpose of this Standard Operating Procedure (SOP) is to provide guidance for the safe operation of centrifuges and rotors in the laboratory. Centrifuges are a commonly used piece of laboratory equipment. Centrifuges spin at high speeds to separate substances with different particle sizes or densities. All centrifuges, including microcentrifuges, can be hazardous if used or maintained improperly.

Types of Centrifuges

There are a number of different types of centrifuges that are found in laboratories. Centrifuges differ based on:

- Physical size
- How fast they can spin
- The ability to keep samples cold or hot
- If they operate under vacuum conditions

Ultracentrifuges, which will be mentioned several times in this SOP, are centrifuges that can reach very high speeds. These centrifuges can accelerate the sample up to 1,000,000 x g.



Examples of different types of centrifuges. They range from small enough to hold in your hand to boxes that can be more than 3 feet per side.



Examples of different types of rotors.

Most centrifuges have a variety of rotors available for them. The rotor used will determine:

- Maximum speed
 - Each rotor has a set maximum speed that must not be exceeded
- Sample density
 - Manufacturers set a maximum sample density for the rotor which must be followed
- Total mass of rotor with samples fully loaded
 - Rotors have a maximum compartment mass that can only be exceeded when the speed the rotor runs at is reduced. The formula for determining reduced speed is:
 - $Reduced\ Speed = Maximum\ Rotor\ Speed \sqrt{\left(\frac{Maximum\ Compartment\ Load}{Actual\ Compartment\ Mass}\right)}$
- The volume and number of samples
- The angle the sample spins at. There are two different types of rotor with regard to angle:
 - Fixed Angle Rotors hold the sample in the same orientation throughout the run, this can be either vertical or at an angle (usually 14 to 40 degrees to vertical).
 - Swinging Bucket Rotors swing out to a horizontal position during centrifugation and return to vertical when the centrifuge stops.

Potential Hazards

Physical hazards: Mechanical stress, metal fatigue and corrosion can lead to mechanical failure over time. Unbalanced centrifugation and other mechanical failures can result in centrifuge explosion¹.

Exposure hazards: Materials being centrifuged can become aerosolized and if the materials are hazardous, this can expose the user to hazardous air when the centrifuge is opened.

Handling Procedures

To avoid accidents and injury, always follow the manufacturer's operating instructions for the centrifuge being used. Make sure rotors are used in the proper centrifuge. (e.g. Beckman rotors should be used only in Beckman centrifuges equipped to handle the specific rotor in question.)

Inspect centrifuge:

Look over the centrifuge and rotor prior to use.

1. If visible, ensure centrifuge spindle is clean.
2. For ultracentrifuges, make sure the classification decal on the centrifuge and rotor match.
3. Check that rotor, safety cups and/or buckets do not have signs of corrosion, cracks or deformities.
4. Ensure centrifuge and rotor are dry.
5. Make sure the rotor is properly seated on the drive shaft.
6. Ensure rotors are properly attached
7. Check that safety cups/buckets are properly seated and able to move freely.
 - o 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.

Prepare samples:

1. Select appropriate tubes or containers for rotor, sample and speed.
 - o Tube/container and rotor bottoms must match. E.g. conical bottom rotors need conical bottom tubes.
 - o Sample must be compatible with tube/container material.
 - o Tube/container must be rated for speed being used.
2. Inspect tubes and containers for cracks or flaws before using.
3. Avoid overfilling or underfilling tubes and containers, make sure to follow manufacturer limits when given.
4. Make sure lids are tightly secured.
5. Balance tubes, see the Balance rotor and containers section below
6. Make sure the exterior of the tubes and/or containers are clean and dry prior to centrifugation.

Balance rotor and containers

The most common cause of centrifuge accidents is user error, especially involving imbalanced rotors. All centrifuge runs must have at least two samples and cannot have only one open space in the rotor.

When balancing rotors remember:

- Both the weight and the density of the materials being centrifuged must be balanced.
- When running a balance tube make sure the contents of the balance is the same as (or as close as possible to) the one inside the sample tube(s).
- Always weigh your containers, with caps on, to ensure they are balanced prior to centrifuging.
- Consult the operator's manual for the centrifuge to determine the amount of imbalance it can withstand.

At 1000 x g, one gram has an effective weight of one kilogram. This means small weight imbalances will have large impacts at high speeds.

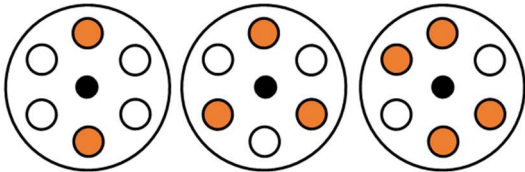
¹ Reports of centrifuge explosions: [Lab Safety Centrifuge Explosions Incidents](#)

Because of their extreme speeds the ultracentrifuges must be carefully balanced.

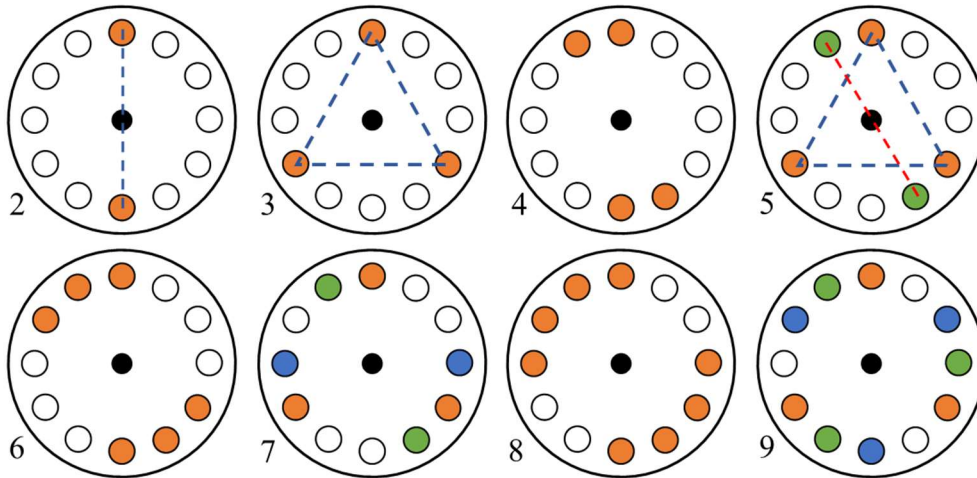
Centrifuges are most easily balanced with containers in pairs, placed directly opposite each other. Three containers can be balanced in circular centrifuges by forming an equilateral triangle.

Examples of balanced rotors

Balanced 6-Position Rotor

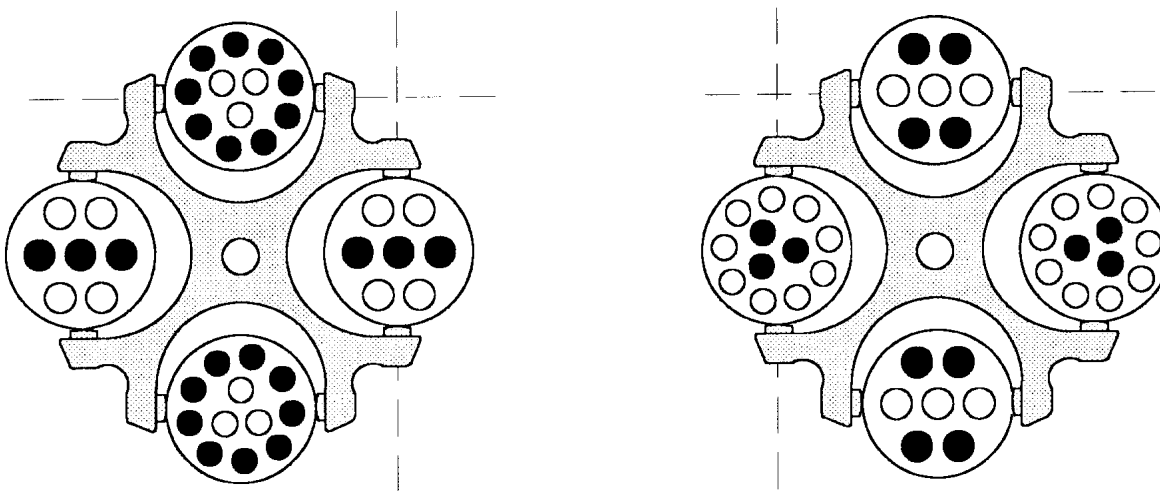


Balanced 12-Position Rotor



Benchtop centrifuges can be balanced with containers in pairs, opposite each other, and triplets, in equilateral triangles, at the same time. See example to the left with 5 containers.

Balanced Swinging Bucket Rotor²



² Image from pdfmedsearch.com/l/laborgeraete-beranek.de1.html

Run centrifuge

- Ensure lid of centrifuge is properly closed.
- Set run speed and time, never exceed the rotor's maximum run speed.
- Do not leave the centrifuge until full operating speed is reached and the machine appears to be running safely.
- Stop the centrifuge immediately if you notice any unusual noises or shaking.
 - Confirm rotor is properly seated and balanced.
 - If problems persist, discontinue use and contact supervisor.
 - Do not use centrifuge until it has been serviced by a qualified technician.
- Make sure the rotor has come to a complete stop before opening the lid.

When centrifuging hazardous materials

Extra precautions should be taken when centrifuging hazardous materials.

- Always wear appropriate PPE for the hazard(s).
- Let the centrifuge stand for at least 10 minutes after the rotor has stopped to allow any aerosols to settle before opening the lid.
- If centrifuging materials from a Biosafety Level 2 or above, rotors must have aerosol containment ("O-rings") or be used inside a biosafety cabinet.
 - Rotors must be loaded and unloaded inside the biosafety cabinet.
- If centrifuging radioactive materials, the centrifuge must be kept behind an appropriate shield.
- If centrifuging hazardous chemicals, open rotor and/or containers inside a fume hood if necessary.

Spills

Check for leaks and spills after each run.

- If you know, or suspect, a spill has occurred, keep the centrifuge cover closed for at least 30 minutes to reduce aerosolization.
- Inspect the sample tubes/containers, safety cups/buckets, rotor and centrifuge.
- If a spill has occurred, use appropriate decontamination and cleanup procedures for the spilled material(s).

Mechanical failure

If the machine experienced problems during a run, turn off the centrifuge immediately and unplug the power cord. Post warning signs so no one plugs the machine back in.

If the machine has a manual lid release lever, the samples can be removed when it is safe to do so. Wait at least 30 minutes before opening the lid.

Do not use the machine until it has been inspected and repaired by a qualified service technician.

Preventive Maintenance

Preventive maintenance is important to keep centrifuges running optimally. These are expensive pieces of equipment and the better they are maintained the longer they will last.

Establish a maintenance schedule:

- Consult the operator's manual or contact manufacturer for information regarding regular servicing.
 - Remember the centrifuge and rotor(s) must be fully decontaminated and cleaned prior to sending for service. A signed statement indicating the centrifuge and rotor(s) are safe must be included with the equipment.
- Clean the interior of the centrifuge and rotor(s) regularly to prevent damage.
 - Do not use alkaline detergents or cleaning solutions that may remove the anodized coating.
 - Use plastic coated brushes to clean the rotor and buckets, do not use ordinary bottle brushes with sharp wire ends.

- Only wash the buckets of a swinging bucket rotor. The body of the rotor should never be immersed to prevent rusting.
- Thoroughly rinse all washed pieces with water and then air dry
- Store rotors and buckets upside down in a dry environment, not inside the centrifuge.
- Lubricate O-rings and threads as recommended by the manufacturer.
 - Note: Rotor life span may be reduced and warranty voided if the rotor is autoclaved. Follow manufacturer recommendations or contact the manufacturer for guidance.

Maintain a log book:

- For all high speed centrifuges and ultracentrifuges maintain records on run dates, durations, speeds, and notes on rotor condition.
 - This will allow accurate information about when a rotor needs to be run at reduced speeds or retired, follow manufacturer recommendations for rotor use.

Cleaning reusable centrifuge tubes and bottles

Appropriate cleaning of reusable tubes and bottles is necessary to prolong their life and avoid having them break or collapse during centrifugation.

To clean centrifuge tubes and bottles:

- Wash with mild detergent in warm water, rinse them thoroughly with clean water and allow them to air dry.
- Avoid cleaning plastic containers in automated dishwashers or glassware washes because they are frequently too hot.
- Autoclave tubes only if absolutely necessary, keep the temperature at or below 100°C and autoclave for 15 minutes.
 - Autoclaving may void the warranty for the container. Check with the manufacturer to make sure autoclaving is safe.

Rotor Lifespan

Some rotors come with an expiration date marked on the rotor or rotor accessories. Rotors and rotor accessories must not be used past their expiration date.

Record warranty periods and retirement recommendations for each class of rotor. Retiring rotors when the warranty expires is prudent.

Online References and Resources

[Centrifuge Rotor Selection and Maintenance](#) by Tammy Goodman

[Respect the Ultra](#) by Jode Plank

[Balancing a centrifuge](#) by Martin Johnsson

University of Nebraska-Lincoln – [Centrifuges: Research or Rocket?](#) – 2008 training video for Care and Handling of Rotors

Revision History		
Rev #	Affected Pages	Change Descriptions
0	All	Release 5/8/2015
1	3, 4	Added: rotor precautions, selection of centrifuge bottles or tubes, prolong tube life and avoid breakage or collapse. 3/28/2016
2	All	Complete revision 10/11/2016