

A centrifuge is an important tool in a research lab. It can also be a dangerous instrument if not used or maintained properly. Most hazards associated with centrifugation stem from one of two sources: mechanical conditions and/or from processing hazardous materials. This SOP discusses both and presents methods for controlling the associated risks.

There are three types of centrifuges in use at URI. Low speed centrifuges that do not exceed 5,000 rpm and are commonly found on bench tops. High speed centrifuges do not exceed 25,000 rpm and are generally floor models. Ultracentrifuges which operate at speeds in excess of 100,000 rpm are freestanding floor models. These are the most expensive and also the most dangerous.

TRAINING

Before using any centrifuge for the first time:

- Review the operator's manual. If a manual is not available in the lab, obtain a copy from the manufacturer or find one online.
- Additionally, the lab supervisor should also provide complete hands-on instruction on how to use the instrument correctly. This instruction should match what you have read in the manual. If it doesn't, ask why it doesn't.
- View the centrifuge safety video from Lab Safety Institute on YouTube <http://www.youtube.com/watch?v=L3MK8Euz3HQ>
- Learn to recognize unsafe centrifuge operations and situations.

METALLURGICAL AND MECHANICAL CONSIDERATIONS

Stress

Centrifugal force puts an extreme load on the rotor that causes stretching or other change in the dimensions of the metal. Rotors are designed to withstand a certain amount of stress and return to their original dimensions. However, if the upper level of stress is exceeded, the rotor will not return to its original size and shape. Minute cracks develop that will cause the rotor to deteriorate over time, leading to possibly dangerous consequences.

To prevent undue stress on the rotor, always:

- Ensure that loads are evenly balanced and density-matched before a run.
- Observe the manufacturer's maximum speed and sample density ratings.
- Observe speed reductions when running high density solutions, plastic adapters, or stainless-steel tubes.

Metal Fatigue

Even when the manufacturer's recommendations are closely followed, rotors can suffer metal fatigue. Repeated cyclical stretching and relaxation changes the metal's microstructure which can result in cracks and eventual failure. Centrifuge manufacturers typically give both an expiration date (the date beyond which the rotor should not be used under any circumstances) and a maximum number of runs.

To prevent mechanical rotor failure due to metal fatigue, observe the following:

- Do not use a rotor past the manufacturer's expiration or safe-service date.
- Keep a rotor-use log to prevent over-use. (Note: newer equipment may have data logging capability. Consult the manufacturer's instructions for specific record-keeping requirements.).

Corrosion

Many rotors are made from titanium or aluminum alloy, metals that are chosen for their advantageous mechanical properties. Although titanium alloys are quite corrosion-resistant, aluminum alloys are not. While a rotor may be made of titanium alloy, other centrifuge components may be made of aluminum or other materials due to design or cost considerations. When corrosion occurs, the metal is weakened and is less able to bear stress from the centrifugal force exerted on it during operation. This combination of stress and corrosion causes the rotor to fail more quickly and at lower stress levels than a non-corroded rotor.

To prevent corrosion, observe the following:

- Select titanium alloy or comparable rotors for areas where corrosive or salts including buffers will be used regularly.
- Alternatively, consider using a rotor made of carbon fiber for superior performance *without corrosion or fatigue*.
- Never clean rotors or associated parts with abrasive wire brushes.
- Avoid using alkaline detergents or cleaning solutions on aluminum parts. (Note: most cleaning solutions designed for decontamination are highly alkaline).

- Minimize exposure of aluminum rotor components to strong acids or bases, alkaline lab detergents, salts (chlorides) or heavy metals (e.g. cesium, lead, or silver). Corrosion will compromise the structural integrity of the rotor.
- If corrosive or alkaline materials have been run or spilled, thoroughly wash affected parts of the centrifuge, rinse well with deionized water, wipe with a towel and allow to air dry.

To Prevent Mechanical Failure

- Maintain a service contract with the manufacturer or other service provider. Service the centrifuge according to the manufacturer's recommendation to ensure the operational integrity of the instrument at all times.
- Observe speed reductions when running high density solutions, plastic adapters, or stainless-steel tubes.
- Use only rotors that are compatible with your centrifuge. Consult the operator's manual for a list of compatible rotors.
- Inspect the rotor before use and after it has been dropped. Do not use the rotor if any cracks, rough spots, pitting, discolorations, or other abnormalities are visible. The PI should contact the manufacturer for service or possible replacement if any of these conditions is observed.
- Never attempt to open the door while the rotor is spinning or attempt to stop the rotor by hand while the rotor is still in motion. *Serious injury may result.*
- Do not move a centrifuge while it is in operation.

Rotor Care and Use

- Each rotor should have a maintenance/usage log to detail the number of runs, hours of use, age of the rotor, rotation speed per run, and dates of servicing.
- Use only the rotor designed for your specific instrument. Using the wrong rotor can cause an explosion in an ultracentrifuge.
- Do not use a damaged rotor. Inspect rotors for wear and/or damage before using. If the rotor appears to be visibly damaged or you know it has been dropped, send it back to the manufacturer for proper evaluation. It may need to be replaced.
- Keep rotors and other exposed parts of centrifuges clean, free of chemicals, chemical residues and infectious materials.
- Metal rotors that are in contact with moisture for extended periods of time may be subject to corrosion and damage. To prevent this, leave the rotor clean and dry after each use. Wash the rotor with mild detergent and rinse well with

deionized water; use a soft nylon bottle brush if necessary. Do not use a wire brush as it may damage the rotor. Dry thoroughly. Check the operator's manual for specific recommendations.

- Both Beckmann and Sorvall recommend taking ultracentrifuge rotors out of service after either 10 years or after the rotor has reached a specified number of runs. If you have an older unit, check with your technical service representative regarding rotor retirement.
- Do not autoclave rotors at temperatures above 100°C.
- Check to ensure that the centrifuge chamber, drive spindle, and tapered mounting surface of the rotor are clean and free of scratches.
- Wipe drive surfaces prior to installing the rotor.
- Make sure rotor, tubes and spindle are dry and that the rotor is properly seated and secured to the drive hub. Do not operate the centrifuge without having the appropriate rotor cover securely fitted, with seals properly in place.
- If the temperature of the chamber is below room temperature, pre-cool the rotor to the lower temperature before securing the rotor. Make sure it is completely dry or it could freeze to the tapered spindle.
- Balance the rotor to within the limits specified (ensure materials of *similar densities, not just similar volumes*, are in opposite positions).
- Do not exceed the design mass for the maximum speed of the rotor. Failure to observe this precaution can result in dangerous and expensive rotor disintegration.
- Do not exceed maximum rotor speed under any circumstance. Speed reduction may be necessary because of weight considerations of tubes, adapters, condition of the rotor, or the density of the solution being centrifuged.
- Excessive vibration of a high-speed centrifuge indicates a grossly unbalanced rotor. Stop the run immediately, remove all samples from the rotor and check counterbalancing in accordance with the centrifuge manufacturer's recommendations. Most high/super centrifuges require counterbalancing within ± 1.0 gram. Ultra-speed, fixed angle rotors require counterbalancing better than ± 0.5 gram. Ask the manufacturer of your centrifuge for the proper counterbalancing procedure.
- Make sure the centrifuge has a safety locking device in place and that it works properly before using the centrifuge. Do not use the instrument if the locking

- device does not work properly or has been inactivated. Have the centrifuge serviced before someone is injured.

Inspection

- Inspect rotors for damage and/or wear before each use.
- The cone area is highly stressed during rotation and should be checked for cracks.
- If there are gouges in the rotor body, the rotor should be serviced. If there are light scratches, check for corrosion.
- Look for corrosion or cracks in the tube cavity.
- Dual row rotors are highly stressed and any damage to these rotors cannot usually be repaired.
- Vertical rotors: check the sealing cap for thread wear. Replace if necessary.
- Swinging bucket rotors: damage to the bucket seat pins cannot be repaired.

When to Derate the Rotor Speed?

- It may be necessary to derate a rotor after it has seen extensive service. Derating is defined as “reducing the maximum safe speed at which the manufacturer recommends using a rotor”.
- Not reducing rotor speed on an older rotor can lead to rotor failure and possible injury to personnel and/or damage to the laboratory.
- Derate the rotor speed whenever:
 - The rotor speed, temperature or a combination of the speed and temperature during operation exceed the solubility of the gradient material and cause it to precipitate; or
 - The compartment load exceeds the maximum specified by the manufacturer; or when a manufacturer recommends, based on the amount of use the rotor has received, limiting the maximum speed at which the rotor is used at some lower level than was listed for the rotor when it was new.

Tube Care

- Before each use, check tubes for cracks and deformities.
- Inspect the inside of cups for rough walls caused by corrosion. Do not use if present.
- Metal or plastic tubes (other than nitrocellulose) should be used whenever possible.

- Make sure each tube compartment is clean and corrosion-free.
- Tubes must be properly balanced in the rotor (0.5 gram at 1G is roughly equivalent to 250 Kg at 500,000G's).
- Use only correctly fitting tubes
- Never fill centrifuge tubes above the maximum recommended by the manufacturer, not to exceed $\frac{3}{4}$ full.

CENTRIFUGING INFECTIOUS MATERIALS

- Centrifugation of infectious materials, including human blood and cell cultures, should be done using sealed rotors, sealed buckets, or a guard bowl cover complete with gasket, as well as safety centrifuge tubes (tube or bottle carrier with sealable cap or "O" ring cap).
- If a spill or leakage is apparent in the centrifuge, disinfect the inside of the unit; disinfect the rotor inside a biosafety cabinet, rinse thoroughly with deionized water, and allowed to air dry completely.
- Do not use abrasive or corrosive materials to clean the rotor as this could damage the rotor permanently.

Follow these recommendations when centrifuging infectious materials:

- Examine tubes and bottles for cracks or stress marks before using them.
- Fill and decant all centrifuge tubes and bottles inside a biosafety cabinet.
- Wipe the outside of tubes with disinfectant before placing in safety cups or rotors.
- Never overfill centrifuge tubes as leakage may occur if tubes are filled to capacity. The maximum for centrifuge tubes is $\frac{3}{4}$ full.
- Use screw cap tubes and cap all tubes securely before spinning.
- Place tubes in safety buckets or sealed rotors. Inspect the "O" ring seal of the safety bucket and the inside of safety buckets or rotors. Open only inside a biosafety cabinet.
- Ensure that the load is properly balanced.
- Never exceed recommended safe rotor speed.
- Stop the centrifuge immediately if an unusual noise or vibration develops.
- Wait 20 minutes for the aerosol to settle before opening the centrifuge. If there is evidence of leakage or tube damage after centrifuging infectious materials, close the lid immediately, allow aerosols to settle an additional 10

minutes, and plan the disinfection procedure following URI's Spill Management Plan.

If a centrifuge malfunctions while centrifuging BSL-2 materials:

- Contact your supervisor or PI to initiate a service call. The unit must be decontaminated completely before it can be serviced.
- Use a hospital-grade disinfectant that is registered with the EPA. Do not use bleach.
- Work with the rotor in a biosafety cabinet. Line the work surface with absorbent matting then carefully spray all surfaces with the disinfectant. Leave on the appropriate length of time (from the label) to properly decontaminate. Rinse with multiple deionized water rinses to remove disinfectant residue.
- When decontamination of the centrifuge has been completed, attach a URI "Notice of Decontamination" label (**Appendix O – Equipment Decontamination Label**).
- To prevent corrosion, do not use bleach or other high or low pH disinfectants if the centrifuge's components are made of aluminum. Consult the operator's manual and follow the manufacturer's recommendation.
- If you think a tube has broken while centrifuging, immediately turn off the unit; do not attempt to open the lid until aerosols have settled. Allow a minimum of 30 minutes before beginning clean-up and decontamination.