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Basic Use of Centrifuges

Contents

General 2
Hazards associated with the use of centrifuges 2
Loading and unloading a rotor 2
Balancing a Rotor: 3
Critical Speed 3
Operation 3
Centrifuging known or potentially infectious materials 4
Use of the brake 4
Precautions 4
Care of rotors 5
Relative Centrifugal Force 5

General

OCDEM possesses a number of bench-top, floor standing and ultra- centrifuges. The following general instructions are a guide to their use. Specific instructions for individual centrifuges will be found in the appropriate instruction manuals. Training will be given on the use of all the centrifuges in the department and no one is to use the ultra-centrifuges without an intensive training session on their use.

The rotor inside a centrifuge can be of two types, fixed angle and swinging bucket, these are not interchangeable between centrifuges, unless they are of the same model and type, but both types may be available for a particular centrifuge.
Horizontal or swinging bucket rotors, where buckets swing out in a horizontal plane when centrifuged, are suitable for most applications, in particular for separating plasma from red cells as the centrifugal force is horizontal, therefore the interface between the plasma and the red cells is at right angles to the tube wall. In contrast the pellet resulting from centrifugation in a fixed angle rotor is deposited at an angle. The main advantage of a fixed angle rotor is that the maximum speed is usually greater, and the time of centrifugation is thus reduced.
Horizontal rotors are provided with buckets which can be filled with a wide range of carriers suitable for different types of tubes and bags. When using such a rotor all the buckets should be fitted even if not in use

Hazards associated with the use of centrifuges

- Mechanical failure of rotating parts (often violent).
- Sample imbalance causing machine movement or stress failure of component parts.
- Contact with rotating parts.
- Sample leaks causing aerosols (possible corrosion, contamination).
- Infection (contact with contaminated components / vapours).
- Electric shock (electric faults in apparatus or cabling / plugs).

Loading and unloading a rotor

- Before placing the rotor in the centrifuge, make sure the bowl is dry and the drive spindle is clean. Do a visual check on the rotor and if there is any sign of corrosion or stress damage report this to the safety officer. Do not use the rotor. Wipe clean all drive surfaces prior to installing the rotor. Lift rotors carefully, keeping your back straight and using your knees to take the weight. Avoid arching your back as far as possible when loading or unloading a rotor into or from a centrifuge. Ask for help if necessary. Likewise, take care when removing a rotor and avoid any jerking action when breaking the contact with the drive spindle.
- Make sure the rotor is seated on the drive hub correctly, and that any securing devices are properly engaged.
- Tighten the rotor securely using the manufacturer’s T-bar.
- Do not overload beyond the rotor's maximum mass.
- When using a horizontal (swing out) rotor, make sure all buckets are hooked correctly and move freely and are not damaged in any way (for example: cracked or pitted).

- Check O-rings on containers and rotors for cracks, nicks or chemical attack. Apply vacuum grease sparingly to the seals of aerosol lids at least weekly.
- Common user errors are failure to secure the rotor to the drive, failure to put the lid on the rotor, and failure to secure the lid.
  In centrifuges that have rotors that are removed and replaced due to tube selection (eg Allegra 64R centrifuges) the user **MUST** check the rotor and 'screw-down' lids are tight before every use using a T-bar where required.
- Double-check to make sure the rotor is not being run beyond its rated maximum. It is good practice to stay at the centrifuge until it is running smoothly at the desired run speed.

Balancing a Rotor:

For a rotor to run smoothly and safely the rotor must be balanced. The following rules apply:

- A rotor must **never** be run with buckets missing although opposite buckets can be left empty.
- All opposing loads must be balanced within a certain weight specified by the manufacturer, taking care that materials of similar densities are in opposite positions in the rotor.
- Loads must be balanced with respect to the pivotal axis of the bucket and the centre of rotation. This can be achieved by the use of balancing tubes.

Most centrifuges are equipped with an imbalance detector which turns the centrifuge off before any damage is done to the rotor. However, this is not the case for incorrect balancing within a bucket. In this case imbalance can result in poor separation and remixing of sedimented material during deceleration. The risk of tube breakage is greatly increased if the bucket is not horizontal at operating speed.

Critical Speed

All centrifuges vibrate at a certain low speed when accelerating and decelerating, this is normal and is called critical speed.

Operation

- Install the rotor, if not already fitted, following the guidelines above.
- Never fill centrifuge tubes above the maximum recommended by the manufacturer.
- Balance tubes and bottles carefully and avoid overfilling (in tubes used in fixed angle rotors, centripetal force drives the fluid up the outside tube wall).
- Check compatibility of tube material to solvent medium and use only correctly fitting tubes.
- Check that the tops of the tubes do not hit the rotor in the horizontal plane before spinning.
- Check to ensure there are no foreign objects present in the centrifuge bowl.
- Once tubes are loaded in the rotor close the lid. **Never** disable lid-locking devices or attempt to open the lid during a run.
- Bring the centrifuge slowly up to the desired speed and remain with it to ensure that the run is proceeding smoothly. If you hear any unusual sounds during this period, stop the centrifuge immediately by switching it off.

- When the run is complete, do not open the door until the rotor has come to a halt; safety lid-locking devices should prevent the lid being opened. If the lid can be opened before the rotor has stopped mark the centrifuge as 'out of use' and call a service engineer. **Never** attempt to slow the rotor while it is still in motion, as serious injury may be sustained.
- Always check for a possible spill. If you find one, be sure to clean both the centrifuge and rotor thoroughly. See *OCDEM SOP S1: Laboratory Rules for the Safe Handling of Blood, Body Fluids and other Human Tissues: Containment Level 2* and *SOP S2: Procedure for handling major chemical spillages, for chemical spills*.

Centrifuging known or potentially infectious materials

- Always choose aerosol containment tubes and rotors when centrifuging infectious or potentially infectious materials.
- Blood must always be centrifuged in sealed aerosol containers.
- Load and unload tubes and rotors in a biological safety cabinet.
- Wait ten minutes before opening the centrifuge door in order to avoid hazardous aerosols.
- If there is evidence of leakage or damage of any kind, close the lid immediately and carefully plan the cleanup.

Use of the brake

The centrifuges in OCDEM are all equipped with a braking mechanism which is normally on. Under normal conditions deceleration will not disturb the sediment, but too severe braking of, for instance, blood bags will create some disturbance between plasma and red cells. If the brake is turned off the centrifuge will take a long time to slow down.

Precautions

Rotor failure during centrifugation causes major damage to the centrifuge, so some simple precautions need to be observed.

- All rotors have a maximum speed with a load of specified weight above which they must not be run. If in any doubt about this consult the manual.
  N.B. If solutions with a specific gravity greater than 1.0 are used, account must be taken of their weight when running at maximum speed. Check in the relevant rotor or centrifuge manual to find out the maximum density that can be spun before adjustment to the maximum speed must be made.
- Spillages **must** be cleaned up at once, liquids spilt on the rotor form an aerosol when the centrifuge is running and can then be dispersed throughout the lab. Particular care should be taken to clean up blood spills as hepatitis and other infectious diseases can be transmitted this way. When cleaning, the bowl, rotor, buckets and carriers must be thoroughly washed. **Do not** use Decon, which is very alkaline and may corrode the metal parts; See the following documents for full instructions:
  - *SOP S4, Disinfection in Containment Level 2 areas*, for cleaning up a blood or body fluid spill.
  - *SOP S2: Procedure for handling major chemical spillages*, for chemical spills.

    - *Local Rules for Laboratory Work with Radioactive Materials* for radioactive spills.
- If an alcohol is used ensure it has completely evaporated before using the centrifuge.
- Breakages, and thus spillages, can be avoided to some extent by prior checking of carriers; i.e. do not use tubes which are cracked or stressed. Should glass be broken in the centrifuge, it must be completely removed before the centrifuge can be used again. A grey dust results from the 'sand blasting' effect of glass in the centrifuge bowl.

Care of rotors

The integrity of rotors can be compromised by corrosion or fatigue. Rotor failure does not have to happen if rotors are properly maintained. Follow the manufacturer's instructions. See *OCDEM SOP E1.1: Centrifuge Rotors and Buckets; Care and Cleaning* for further instructions. Some general guidelines are:

- Keep rotors clean and dry. Wash immediately if spills occur or if salts or other corrosive materials have been used. Dry the rotor thoroughly and store upside down with the cover and inserts removed.
- Be gentle with aluminium rotors. Avoid harsh detergents or bottle brushes with sharp wire ends especially when cleaning aluminium rotors. Finish rinsing with de-ionised water.
- Inspect rotors regularly. If there are rough spots, pitting, white powder deposits, or heavy discolouration, do not run the rotor. Have it checked by the manufacturer's representative.
- Maintain rotor logs. Eventually every rotor must be retired and as ultra rotors age, their maximum speed must be derated. It is imperative to keep diligent logs.

Relative Centrifugal Force

This can be calculated for any speed at which the centrifuge is run from the following equation:

$$RCF=1.12r(r.p.m./1000)^2$$

Where r is the radius of the rotor in mm.

Centrifugal runs can be duplicated in different centrifuges by calculating the RCF for different rotors, this can be simplified by reference to a published nomogram.

Review History				
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1	01/10/2003	New Document	Author KNF/SMH	Oct 2005
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1a	Oct 2006	Rotation of chair of Academic Committee and BSO	SMH	Oct 2008
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