Centrifuge Biosafety

Brian Petuch, CBSP, RBP

The Usual Photo and Story

INCIDENTS & OBSERVATIONS

External Centrifuge Incidents

- Tufts University 2006
  - Botulinum toxin exposure
  - Broken tube
  - OSHA violation
    - Emergency procedures
    - Training
    - PPE
- University Texas 2006
  - Genetic cross of H5N1 & H3N2
  - Cracked cap leaked fluid into rotor and bowl
  - Centrifuge in open lab
  - BSL-3 w/ respiratory PPE
  - Postdoc placed on Tamiflu

Centrifuge is #1 Contaminated Instrument in rDNA Lab

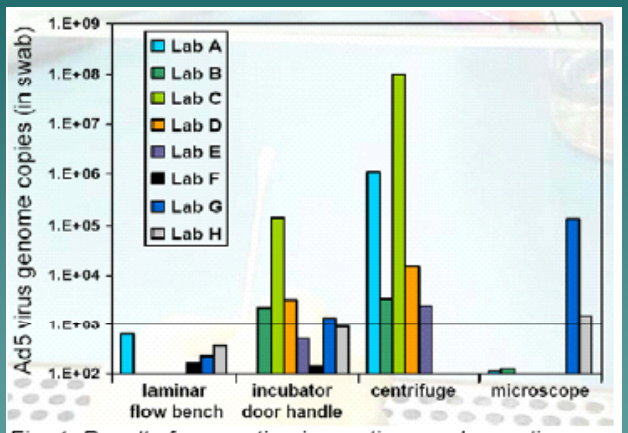


Fig. 1. Results from routine inspections and sampling.

Sampling for AdV5 strains in 5/8 Swiss labs showed significant contamination throughout the lab (Figure 1). (Guido Vogel, Biological and Chemical Safety Administration, Basel, Switzerland. ABSA Annual Meeting, October 2003)

Information Courtesy of ThermoScientific

CAUSES OF ROTOR FAILURES

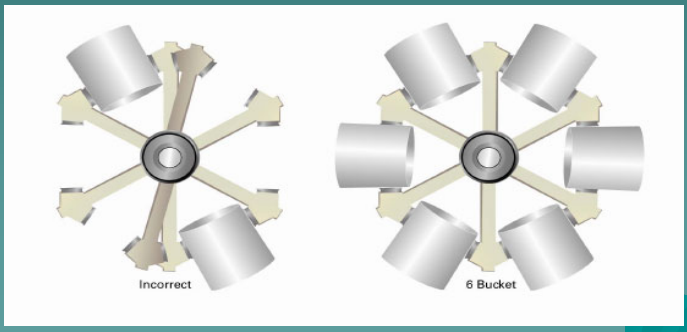
Six Causes of Rotor Failure

1. Incorrect Loading or Balancing
2. Incorrect Attachment
3. Consumable Failure/Biocontainment
4. Overloading
5. Corrosion
6. Fatigue

Rotor Failure Cause 1

Preventing Incorrect Loading

- Always run centrifuge with ALL buckets in place
- Always load buckets symmetrically
- Always balance adjacent and opposing loads within manufacturer's recommended tolerance



Rotor Failure Cause 2

Ensuring Proper Attachment

- **Attachment to Centrifuge**
  - Superspeed rotors must be locked down to centrifuge drive by tightening the lock knob
  - Low speed rotors must be fastened by locking pin
  - General purpose, small benchtop, and micro-centrifuges must be fastened by locking nut or other mechanism as specified by manufacturer

Always double check that your rotor is locked down before pressing start !

Rotor Failure Cause 2

Ensuring Proper Attachment

- **Swinging Bucket Rotors**
  - Buckets must be placed properly on the swinging arms
  - Swinging bucket rotors must have all buckets in place
  - Buckets should be cleaned and lubricated regularly to ensure a free swing out during the run

For swinging bucket rotors, gently swing out each bucket to ensure a free easy motion. Always double check before pressing start

Ensuring Proper Attachment

A Thermo plate carrier was used with a Beckman yoke. The clearance seemed OK, until the centrifuge accelerated. The metal components flexed enough to force the carrier off the swing pins, into the chamber armor.

The centrifuge was destroyed, costing >$38,000. And a spill of pathogen occurred.

Ensuring Proper Attachment

A difference of +0.8 cm resulted in a destroyed centrifuge.

DO NOT MIX COMPONENTS BETWEEN MANUFACTURES!!!!

Rotor Failure Cause 2

Ensuring Proper Attachment

Swinging bucket rotor failed while spinning human blood. Bucket and trunnion chunk lifted the lid, spewing blood around the lab.

Rotor Failure Cause 3

Preventing Consumable Failure/ Biocontainment

Preventing Consumable Failure

- Ensure you are not exceeding the maximum rated speed by the manufacturer
- Ensure consumable is chemically compatible with your sample
- Make sure you have all the parts and use according to the directions
- Know the history of shared consumables
- Inspect before every use and do not use if signs of wear are apparent
- Implement replacement program – consumables should be replaced regularly
- If in doubt contact manufacturer and verify proper use

Biocontainment

- Biocontainment Certified* Lids for Swinging Buckets to enclose any kind of tubes
- Individual Biocontainment Certified* Tube Enclosures – would provide double enclosure under the Biocontainment Certified Lids
- Biocontainment Certified* Rotor Lids for Fixed Angle Rotors

*Biocontainment certification by CAMR in Porton Down, UK

Preventing Consumable Failure/ Biocontainment

Rotor Failure Cause 3

- Overfilling / Under filling
- Seal O-ring (if needed) must be present and in good condition
- Proper Tube Material & Chemical Resistance
  - Polypropylene
  - Polyethylene
  - Polycarbonate

Preventing Consumable Failure/ Biocontainment

Rotor Failure Cause 3

O-ring (missing or damaged)

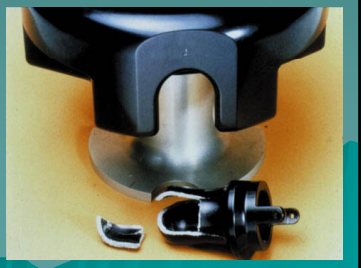
Rotor Failure Cause 4

Preventing Overloading

- All rotors are designed with a maximum load tolerance at the maximum speed
  - If the maximum load is exceeded then the maximum speed MUST be derated – ALWAYS check this in the **manual** and use the formula below.

- Ultraspeed swinging bucket rotors used for gradient work – density of sample should be verified against speed and temperature to prevent point load failure

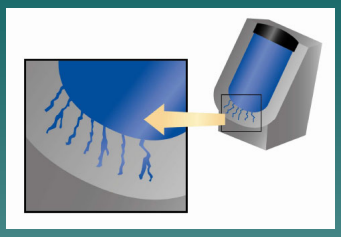
$$\text{Reduced Speed (rpm)} = \text{Maximum Rotor Speed (rpm)} \times \sqrt{\frac{\text{Maximum Compartment Mass}}{\text{Actual Compartment Mass}}}$$



Rotor Failure Cause 5

Preventing Corrosion

- Surface corrosion - caused by long term exposure to moisture or chemicals
- Stress corrosion - surface corrosion that is allowed to propagate until microcracks are formed deep into the structure of the rotor
- How to prevent surface corrosion:
  - Clean rotors between every use
  - Thoroughly dry rotors between uses
  - Remove adapters between runs
  - Never use metallic objects to open or remove parts
  - Always use cleaning, disinfection and sterilization agents that are chemically compatible with the rotor



If your application is corrosive consider an alternative rotor material such as carbon fiber!

Rotor Failure Cause 5

Preventing Corrosion

- Rotor used with improper tubes, closures and high salt solution
- Tubes are frozen into the rotor
- This rotor is a total loss!

Rotor Failure Cause 6

Preventing Fatigue

- Rotors are exposed to high g-forces and do wear out
- The molecular structure of the rotor changes as the metal elongates repeatedly – **eventually they will fail!**
  - Ultraspeed rotors have a recommended use and retirement span rated by the number of cycles or hours used
  - Superspeed rotors are warranted for 10 years and should be considered for retirement even if they are in good condition
  - All other rotors – the guidelines vary and should be verified before use
  - All rotors need to be retired – even when they appear to be in good appearance – metallic parts fatigue over repeated uses with no visible signs of wear

Rotor Failure Cause 6

Preventing Fatigue

- *Have your rotors inspected regularly by a trained technician!*
- *Rotor instruction manuals must be available for users; they provide detailed information on operation, derating and use/care*
- *Log books are required for ultraspeed centrifuges!*
- *Log books are needed to meet manufacturer's warranty for high and low speed rotors*

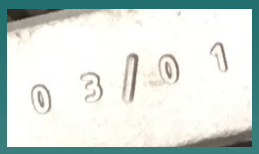
Rotor Failure Cause 6

Preventing Fatigue

This is the rotor manufacture date

Picture dated 4/20/2017

Preventing Fatigue



This is the rotor manufacture date.

According to table, the rotor was beyond useful life after 2008.

Centrifuge 5804/5804 R/5810/5810 R – Operating manual

Rotor	Maximum service from commissioning onward	
A-2-DWP-AT	100,000 mechanical cycles	7 years
A-2-DWP	34,000 mechanical cycles	7 years
A-4-44	34,000 mechanical cycles	7 years
A-4-62	40,000 mechanical cycles	7 years
A-4-81	100,000 mechanical cycles	7 years
F-34-6-38	75,000 mechanical cycles	7 years
FA-45-6-30		7 years
FA-45-48-11	75,000 mechanical cycles	7 years
FA-45-20-17	75,000 mechanical cycles	7 years
F 35 48 17	75,000 mechanical cycles	7 years
S-4-72	60,000 mechanical cycles	7 years
S-4-104	100,000 mechanical cycles	7 years
T-60-11		7 years

Accessories	Maximum service from commissioning onward	
Aerosol-tight rotor lid, without replaceable seals	50 autoclaving cycles	–
Rotor lid QuickLock		3 years
Seals of the QuickLock rotor lid	50 autoclaving cycles	–
Rotor lid and caps made of polycarbonate (PC), polypropylene (PP) or polyetherimide (PEI)	50 autoclaving cycles	3 years
Adapters	–	1 year

Summary – Be Smart, Be Proactive!

- Improve the life of your rotor and prevent damaging failures:
  - Balance properly
  - Attaching rotor and parts correctly
  - Proactively managing consumable use
  - Never overload
  - Prevent corrosion with best lab practices
  - Retire rotors

Improve your lab experience by preventing messy spills or catastrophic accidents that cause potential exposures, significant repair costs, and loss of your precious samples!

RISK ASSESSMENT TOOLS

Risk Assessment

Experiment 8: centrifuge spill without rotor seal in place
An outdated Sorvall GSA rotor had its 'O' ring seal removed and had 10 ml of a 5×10^9 spore suspension gently pipetted into the rotor chamber. The rotor was accelerated to 4000 rev min^{-1} in a RC5B centrifuge, braked and the centrifuge lid opened while the air samplers were operated.

Experiment 9: unsealed bucket on a swing-out centrifuge rotor
A set of sealed rectangular centrifuge buckets with screw down lids was tested to find out if they generated microbial aerosols. It was found that these buckets were contained when the bucket seal was in place and applied with silicone grease supplied by the manufacturer. However, aerosols were generated when the seal was not in place. In experiment 9a the bucket contained two overfilled Falcon centrifuge tubes containing a 9×10^9 spore ml^{-1} suspension and in experiment 9b a 50 ml spill of the same suspension was 'rolled' around inside the bucket so that some of the fluid would rest on the inside walls of the lid before centrifugation.

A. Bennett & S. Parks, Appl Micro 100 (2006) 658-663

Risk Assessment

	Accident	Casella (CFU m^{-3})	Andersen (CFU m^{-3})	Cyclone (CFU m^{-3})
1	Dropping a flask	173	643	$1{\cdot}03 \times 10^3$
2	Dropping a Thompson bottle	$2{\cdot}48 \times 10^3$	$3{\cdot}48 \times 10^3$ *	$1{\cdot}37 \times 10^4$
3	15 ml spill	387	493†	$2{\cdot}07 \times 10^3$
4	Dropping three bottles	588	$1{\cdot}06 \times 10^3$ *	$3{\cdot}98 \times 10^3$
5	Peristaltic pump	634	886	$5{\cdot}18 \times 10^3$
6	Syringe filter	$3{\cdot}70 \times 10^3$	$3{\cdot}43 \times 10^3$†	$1{\cdot}77 \times 10^4$
7	Fungal plate	$>3{\cdot}3 \times 10^3$	$1{\cdot}34 \times 10^5$ *	$>1{\cdot}56 \times 10^5$
8	Centrifuge spill	$>3{\cdot}3 \times 10^3$	$1{\cdot}71 \times 10^4$†	$2{\cdot}30 \times 10^4$
9a	Centrifuge bucket	150	64	142
9b	Centrifuge bucket	$3{\cdot}00 \times 10^3$	$1{\cdot}10 \times 10^3$ *	$1{\cdot}50 \times 10^3$
10	Bacterial plate	26·7	3·6	8·2
11	Orbital shaker	$1{\cdot}15 \times 10^3$	818*	871

*Over 50% of particles less than 2·2 μm.
†Over 90% less than 2·2 μm.
‡50% less than 1·5 μm.

Risk Assessment

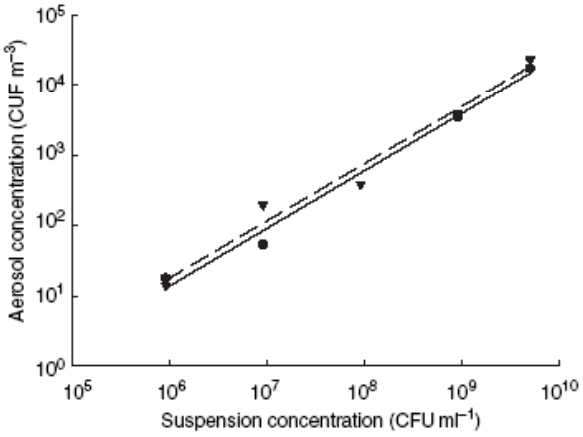


Figure 2 Aerosol generated from centrifuge accident (●, Andersen: $y = x^{0{\cdot}82}/5828$, $r^2 = 0{\cdot}989$; ▼, Cyclone: $y = x^{0{\cdot}81}/4169$, $r^2 = 0{\cdot}975$).

Risk Assessment

Table 3 Spray factors obtained from accidents

Type of accident	SF ($\times 10^6$) ml m^{-3}
Smashed flask (50 ml)	0·52
Smashed Thompson bottle (300 ml)	6·85
Spill of 15 ml	1·04
Three bottle drop	1·99
Blocked peristaltic pump	2·59
Blocked syringe filter	8·85
Centrifuge rotor spill	4·60
Centrifuge bucket spill	0·17
Shaking Incubator	1·28

SF, Spray factor.

Spray Factor, coupled with breathing rate and lab air changes can be used to estimate employee dose

RESOURCES

Resources

- Manufacture's Instruction Manuals
- Rotor Logs for Derating
- Beckman Rotor Safety Guide
- Beckman Biosafe System Guide
- Eppendorf Aerosol-Tight Guide
- Thermo Rotor Care Guide
- ThermoLite Fiber Rotor Guide
- VWR Microcentrifugation Guide