



# Training on *Mycobacterium tuberculosis* drug susceptibility testing (MGIT DST – Liquid Method)

## Module 5: Essential equipment in a TB Culture Laboratory

Date:

Uganda Supranational Reference Laboratory

# Outline

- Guidelines for all equipment in a culture laboratory
- Biosafety cabinet
- Incubators
- Centrifuge



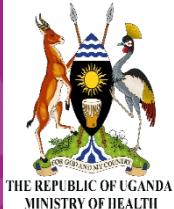
# Main equipment needed for a MGIT TB culture

- Biological safety cabinet
- Safety centrifuge
- Incubator
- Refrigerator
- Autoclave
- pH meter
- Microscope
- Thermometer
- Vortex
- Orbital shaker
- Balance
- BACTEC™ MGIT™ 960 machine



# General requirements for all equipment

- SOPs developed for equipment, read and followed
- Manuals should be readily available for review by staff
- A maintenance schedule, as required by the manufacturer, should be established
- Any corrective action or repairs should be documented
- Proper inventory system



# Definition of a Bio Safety Cabinet(BSC)

- A BSC is a ventilated, enclosed cabinet
- The BSC is the most important piece of equipment in a mycobacteriology laboratory for the primary containment of biological hazards.



# Why BSCs are essential

When properly installed, used and maintained, BSCs will:

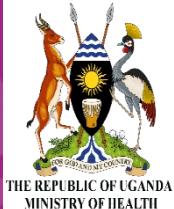
- Reduce laboratory-acquired infections during work on hazardous micro-organisms
- Protect the laboratory environment from hazardous microorganisms like infectious aerosols generated during active handling of liquid materials.
- Minimize contamination of cultures



# Safety features of BSC's

- High efficiency particulate air (HEPA) filters
- Airflow patterns
  - Air curtain at opening
  - Laminar flow of filtered air inside
- Exhaust system
  - Filtered air exhausted

*Note: Safe use requires appropriate operator technique*



# BSC HEPA filters

Constructed of paper-thin sheets of borosilicate fibres

Pleated to increase surface area and affixed to a frame

99.97% effective in removing particles as small as 0.3 µm in diameter

Highly effective in trapping TB bacilli (each ~ 2–10 µm long and 0.4 µm wide)

After filtration, microbe-free air is exhausted from the BSC

## HEPA Filter



[www.airfilterexchange.co  
m](http://www.airfilterexchange.com)



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# BSC airflow and exhaust

## Airflow patterns

- Air is drawn into the cabinet through the front opening by the fan motor
- Direction and velocity of airflow in the cabinet varies for different classes of cabinets

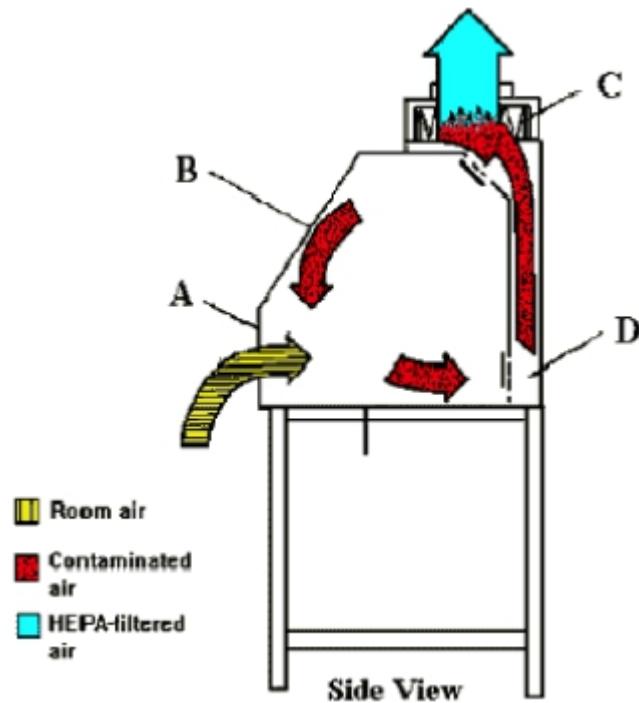
## Exhaust system

- Exhaust of air from the cabinet
- Exhaust of room air drawn through cabinet
- May contribute to negative airflow in the TB culture room

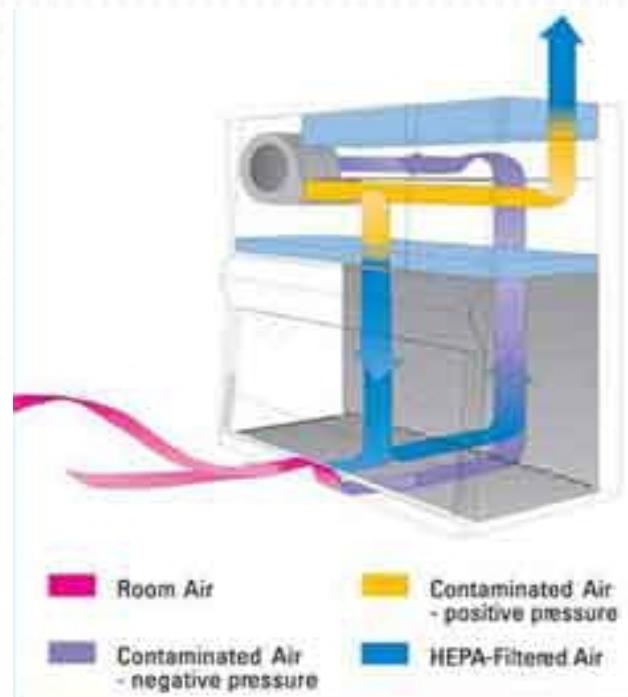


• Accessory fan needed to pull air exhausted from cabinet to outside

# BSC airflow and exhaust



Class I



Class II

# BSC classes I and II

Type	Face velocity (lfpm)	Airflow pattern	TB precaution level	Product protection
<b>Class I open front</b>	75	In at front, rear and top through HEPA filter	Moderate, High	No
<b>Class II Type A2</b>	75	70% recirculated through HEPA; exhaust through HEPA	Moderate, High	Yes
<b>Type B1</b>	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Moderate, High	Yes
<b>Type B2</b>	100	No recirculation. Total exhaust via HEPA and hard ducted	Moderate, High	Yes
<b>Type B3</b>	100	Same as IIA but plenum under negative pressure to room, and exhaust air is ducted	Moderate, High	Yes



# Comparing levels of protection

Primary barrier	Personnel	Product	Environment
BSC Class I	X		X
BSC Class II	X	X	X
Fume Hood	X		

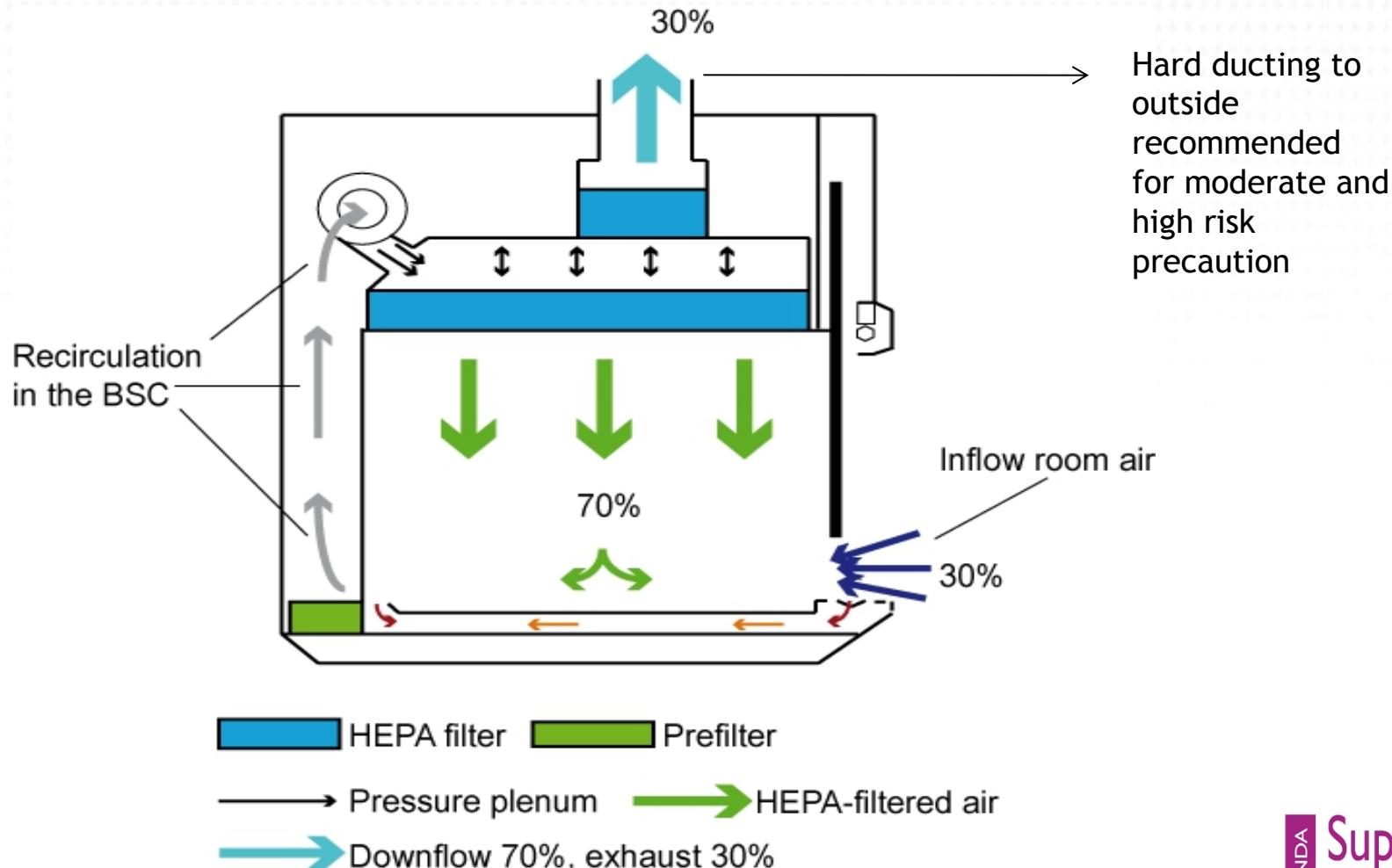


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# Class II A2 biological safety cabinet: Airflow pattern



# BSC Containment can be compromised by;

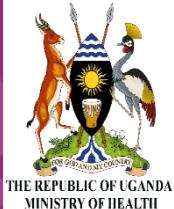
- Poor location in room – area too busy
- Room air currents
- Open windows
- Decreased airflow inside BSC
- Leaking or blocked HEPA filters
- Poor maintenance of cabinet
- Poor user technique
- Crowded work surfaces
- Bunsen burner and continuous flame (vs. Flame boy Bacti-cinerator)



# BSC: Certification

## Certification of each BSC

- Functional operation and integrity of BSC is ensured
- Must be performed by qualified service technicians
- Certification must be performed
  - a) At installation,
  - b) Regularly at least once per year
  - c) Whenever BSC is moved
  - d) After repair



# BSC fan

## Appropriate use of BSC fan

- Switch fan on for at least 15 minutes prior to use
- Creates airflow and air barrier in the BSC
- Leaving the BSC fan on at all times is recommended to help maintain negative pressure in the procedure room
- Allow the BSC to stabilise according to manufacturers instructions



# UV light

## Appropriate use of UV light

- Turned on for a minimum of 30 minutes at the end of working day
- Must be dust-free to be effective
- Manufacturers recommend changing every 3-6 months
  - If used, it must be at a maximum of 30 cm from exposed surface
- UV light is **not** required for the safe operation of BSC



# BSC: Maintenance

## Routine maintenance

- Check the airflow on the indicator gauge
- Record the gauge reading daily, at installation and after maintenance on BSC
- Look for a reading on the gauge outside of the specified range
  1. Drastic increase in reading – filter clogged and needs to be changed
  2. Drastic decrease in reading – hole in filter



# BSC Maintenance

- Call service company to change HEPA filters and record on corrective action log
- Record airflow reading on log sheets
- Check alarm and record result
- Check the direction of airflow with tissue taped to edge of opening
- Weekly smoke test



# BSC Maintenance

## Cleaning work surfaces inside BSC

- Disinfect the work surface, interior walls and interior window surface before and after work
- Wipe clean with sterile water if bleach or other corrosive agent was used as a disinfectant
- Record cleanings on log sheets



# BSC daily maintenance log

National Tuberculosis and Leprosy Control Program  
National TB Reference Laboratory

Code: E008 F2	Version: 1.0	Effective date: 01-Dec-17	Authorized by: Lab Man	
<b>Biosafety Cabinet cleaning Log</b>				
CTRL/BSC/ CWRU/006	SERIAL #: 6S-15-B-7619	Model: BBF 6SS		
Location: BSL3				
<b>MAINTENANCE:</b> Clean before and after with 5x Lysol/1% Bleach followed by 70% Ethanol				
February 15				
DAY	Before	After	TECH	Comments
1				
2	WE	WE	WE	
3	WE	WE	WE	
4				
5				
6				
7				
8				
9	WE	WE	WE	
10	WE	WE	WE	
11				
12				
13				
14				
15				
16	WE	WE	WE	
17	WE	WE	WE	
18				
19				
20				

Example of log sheet for recording daily maintenance for BSCs.



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# Laboratory work performed in a BSC

- Homogenization and decontamination of specimens
- Opening specimen or culture
- Opening centrifuge buckets
- Decanting processed specimens
- Inoculating media
- Preparing smears from cultures
- Identification test procedures
- Drug susceptibility testing



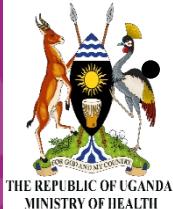
# Preparing for work in the BSC

**Collect all supplies needed prior to working inside the BSC**

- Minimize airflow disturbances during work
- Avoid overcrowding or clutter in the BSC

**Use checklist to collect supplies**

- Personal protective equipment
- All consumables
- Reagents and media
- Disinfectants
- Specimens



# TB culture checklist example

TB Culture Check List		
Date:		
Initials:		
Equipment and Supplies	Yes	
1 BSC, Class II, Fan turned on		
5 Safety Bunsen Burner or Flame Boy		
6 Centrifuge with refrigeration unit on		
7 Centrifuge tubes 50 mL conical		
8 Rack (30 mm)		
9 1.0 ml pipette		
10 Pipette aide		
11 Sterile transfer pipettes		
12 Automatic pipette		
13 Filter pipette tips, 100µl, 1000µl		
14 Disinfectant, freshly made		
15 70% alcohol		
16 Cotton		
17 Waste container for pipettes		
18 Waste container for disposables		
19 Waste container for solutions		
20 Vortex mixer		

21	General lab glassware
22	Slide warmer
23	Balance
24	Stainless steel tray
25	Timer
26	Gown
27	Gloves
28	N95 respirator
29	sharpies
30	labels
31	Slides
32	Pencil
33	Funnels
Reagents and Media	
1	Sodium Hydroxide (NaOH) solution 4%
2	Sodium Citrate solution
3	Phosphate Buffered Saline (PBS)
4	LJ
5	BD MGIT Tubes
6	PANTA
7	Tubes distilled water
8	NALC



# Set-up of BSC workstation

Place absorbent paper soaked with disinfectant on the work surface

Limit the number of items in the cabinet

Do not block any of the air grills – front or back

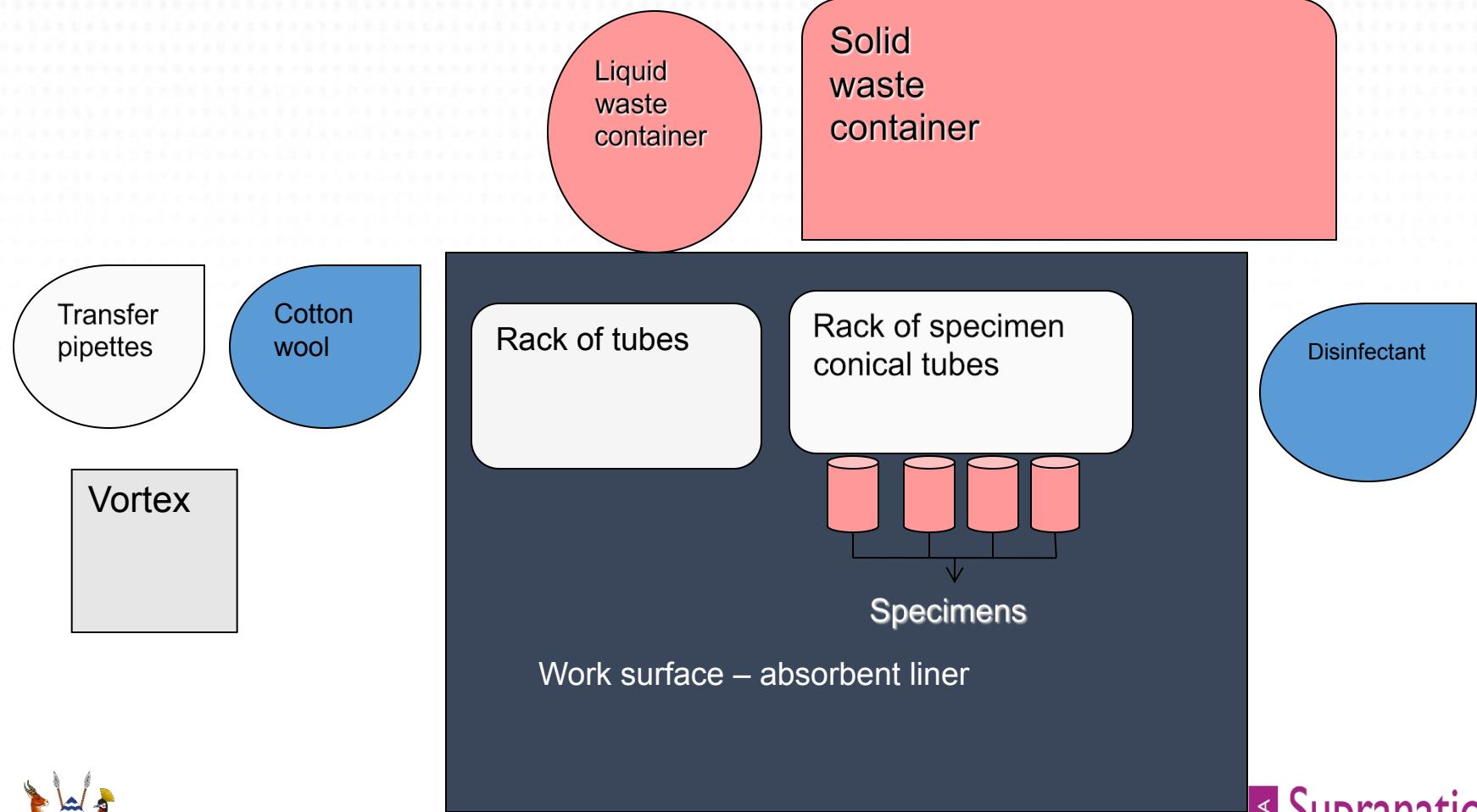


NO



YES

# BSC placement



# Use of BSC

- Perform all operations at least 10 cm from the front grill on the work surface
- Place materials and supplies so as to avoid movements of dirty items over clean items



# Completing work in BSC

- After work is completed, remove the waste bag from BSC and appropriately dispose
- Wipe down the inside of the BSC and work surface with a mycobactericidal disinfectant
- Run fan for at least 10 minutes after completing work



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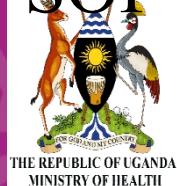
Timely Accurate Diagnostics for a TB-Free Africa

# Fumigation of BSC

BSCs must be decontaminated using Potassium Permanganate and formaldehyde before filter changes, after major spills, and before moving BSC to another location

- HEPA filters must be decontaminated prior to removal from the cabinet

Fumigation and filter changes should be performed by qualified service technicians following an established SOP



# Safety centrifuge

Used in the mycobacteriology laboratory for concentrating bacterial cells following processing of sputum or other specimens

Rotor: component that is rotated by the drive system while holding the containers being centrifuged

## Safety centrifuge cup

- Air- and liquid-tight seal
- Holds specimen tubes
- Provides containment for aerosols that may be released during centrifugation



# Issues with use of safety centrifuges

## Aerosol generation hazards

- Aerosols may be generated when tubes containing liquids break or leak during centrifugation
- Critically important to use safety buckets as secondary containment
- Safety buckets must be opened in the BSC

## Mechanical failure

- Broken drive shaft
- Faulty bearings

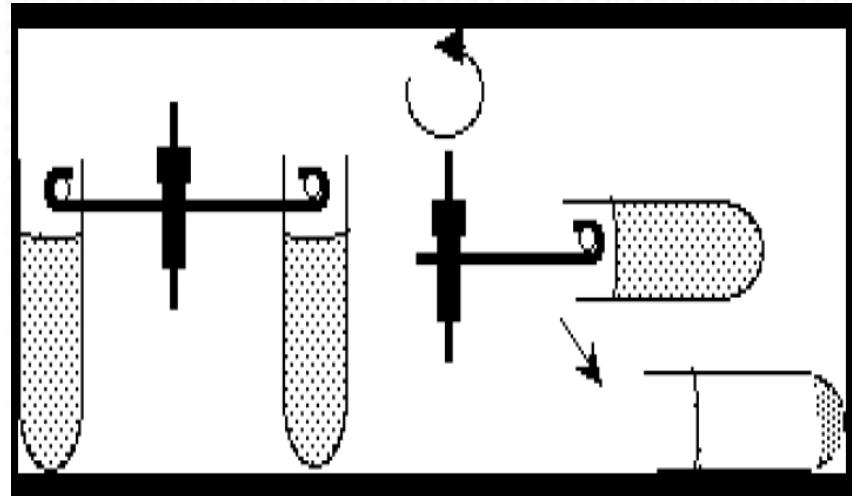
Centrifuges must be well maintained



# Swinging bucket rotors

## Advantages

- Longer distance of travel allows better separation
- Easier to withdraw or decant supernatant without disturbing pellet



# Centrifuge recommendations for mycobacterial specimen processing

- Seal centrifuge buckets tight (for safety!)
- Refrigerate (at 4 °C) to reduce the percentage of mycobacteria killed due to heat generated during centrifugation
- Required G force: 3000 x g (not RPM)



# Centrifuge: RPM vs RCF (g force)

RPM = revolutions per minute

These are not the same

RCF = relative centrifugal force or g force

Setting the speed or g force

- Some centrifuges can be set at both RPM and RCF
- Older centrifuges can only be set at RPM
- Need to use formula or graph to convert RPM to RCF ( $3000 \times g$  required)



# Calculating RPM required to reach desired RCF

**Formula:**

$$\text{RPM} = 1000 \times \sqrt{\text{RCF}} / 1.12 \times r$$

$r$  = radius in mm from the centrifuge spindle to bottom end of tube

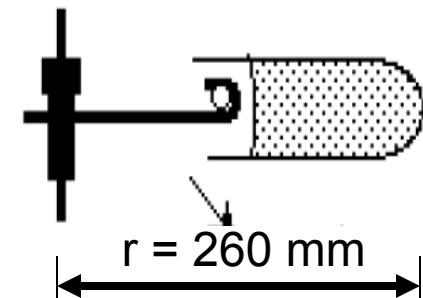
$\text{RCF}$  = desired centrifugal force

**Example:**  $r = 260$  mm  $\text{RCF} = 3000 \times g$

$$\text{RPM} = 1000 \times \sqrt{3000} / 1.12 \times 260$$

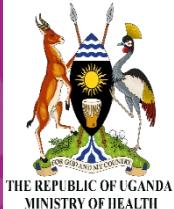
$$\text{RPM} = 1000 \times \sqrt{10.3} = 1000 \times 3.2$$

$$\text{RPM} = 3200$$



# Before use of the centrifuge:

- Inspect the interior of the bowl for stains
- Inspect rotors and buckets for cracks and signs of corrosion
- Check log book to ensure centrifuge is in good working condition
- Pre-cool centrifuge prior to use upto 4 °C



# Use of safety centrifuge

Centrifuge load **MUST** always be balanced to avoid risk of damage.

- Add a balance tube with same volume of water if processing an odd number of specimens

Select the required time and g force or RPM

Stop centrifuge immediately if any abnormal noise and/or shaking is noticed

Never use the brake to stop a normal operating centrifuge for TB specimen processing



**ALWAYS** open the sealed buckets inside  
the BSC

# Tubes for use in safety centrifuge

Select type of tube based on:

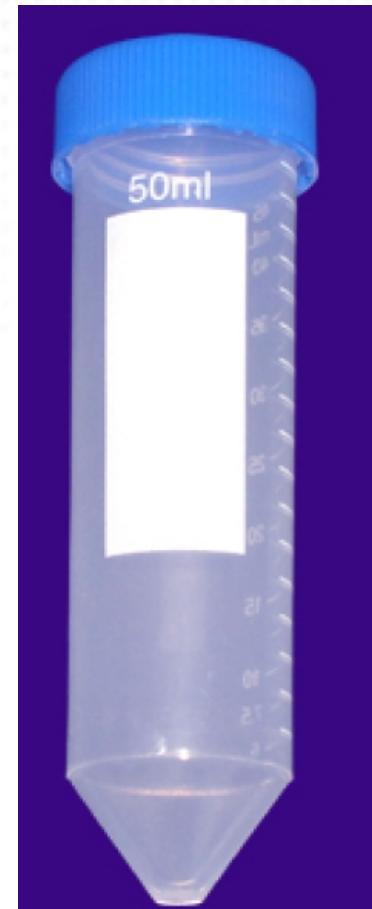
- G force used for swing-out bucket
- Time of centrifugation
- Temperature
- Compatibility with specimen
- Chemically inert

Visually inspect tubes

- Do not use if tubes have:
  - Cracks
  - Scratches
  - Chipped rims

Fill tubes only to recommended volume

- Do not overfill tubes
- High g forces can drive liquid up to the cap and cause overflowing



# Maintenance of safety centrifuge

## Weekly

- Disinfect buckets and lids
  - Autoclave metal parts at dry heat 15 minutes cycle OR soak in disinfectant (not bleach or alcohol) for 15 minutes, rinse with sterile water and air dry
  - Soak lids in freshly prepared 1% bleach for 5 minutes and rinse with 70% alcohol or sterile water

## Monthly

- Clean the centrifuge housing, rotor chamber, rotors and rotor accessories with a neutral cleaning agent, e.g. mild soap
- Lubricate rotor threads with lubricant
- Lubricate O rings with lubricant supplied by manufacturer
- Clean plastic and non-metal parts with a fresh solution of 10% sodium hypochlorite (bleach), followed by a rinse with sterile water



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# Maintenance of safety centrifuge

Full service should be provided by a qualified service technician, which may include the following:

- Centrifuge brushes
- Timer
- Electrical components
- Leaks
- Speed



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# Incubators



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# Routine maintenance

## Record temperatures daily

- Use a separate thermometer stored in a sealed glycerol container
- Calibrate thermometer every year

Clean with disinfectant immediately after any infectious spills

Clean thoroughly with disinfectant on a monthly basis



Walk in incubator, Osong, South Korea

# Use of incubators

## Keep door(s) closed

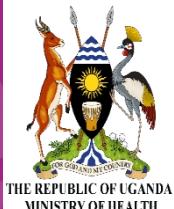
- Prevents heat loss
- Keeps temperature stable

## Do not place containers of media too close together

- Space is needed for adequate air and temperature circulation

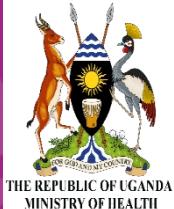
## Label racks with week of incubation

- Enables efficient checking for growth every week for the 6–8 week incubation period



# ASSESSMENT

- List all the important equipment required in a TB Culture Laboratory.
- Describe the maintenance measures for each of the equipment listed above.



# Summary

Each instrument requires specific knowledge to calibrate, operate and maintain properly

Procedures must be followed exactly

- To ensure safety in the laboratory
- To ensure proper functioning of the instrument

Usage logs, corrective action logs and maintenance records must be recorded in log books kept for each instrument

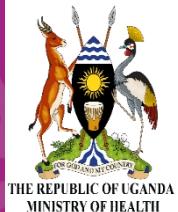


# References

- GLI TB training package  
<http://www.stoptb.org/wg/gli/trainingpackages.asp>
- [www.who.int/tb](http://www.who.int/tb)
- <http://www.who.int/tb/publications/molecular-test-resistance/en>
- <http://www.who.int/tb/dots/laboratory/policy/en>



# Acknowledgement



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