THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 9: Essential equipment in a TB Culture Laboratory

Outline

- General rules for all equipment
- 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
  - Used during work on hazardous micro-organisms
  - Protects both operator and environment from exposure to infectious aerosols generated during active handling of liquid materials

- 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
- Minimize contamination of cultures
- Protect the laboratory environment from hazardous microorganisms

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

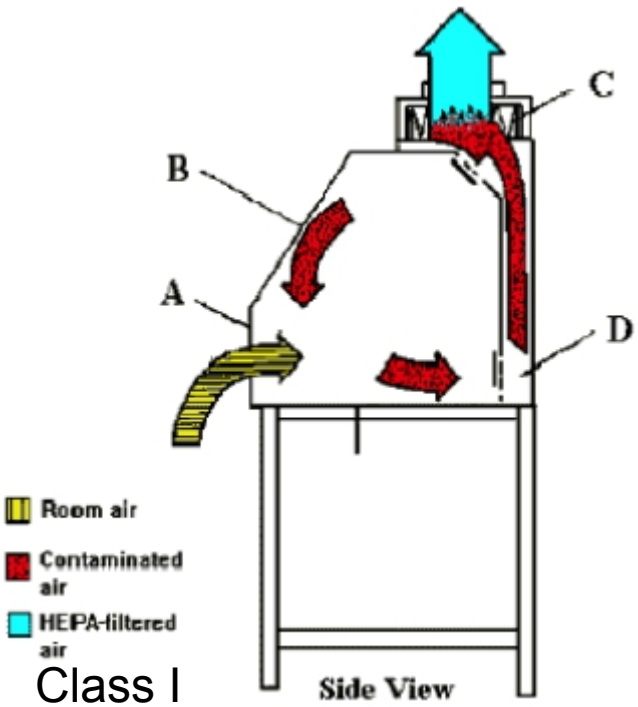
www.airfilterexchange.com

BSC airflow and exhaust

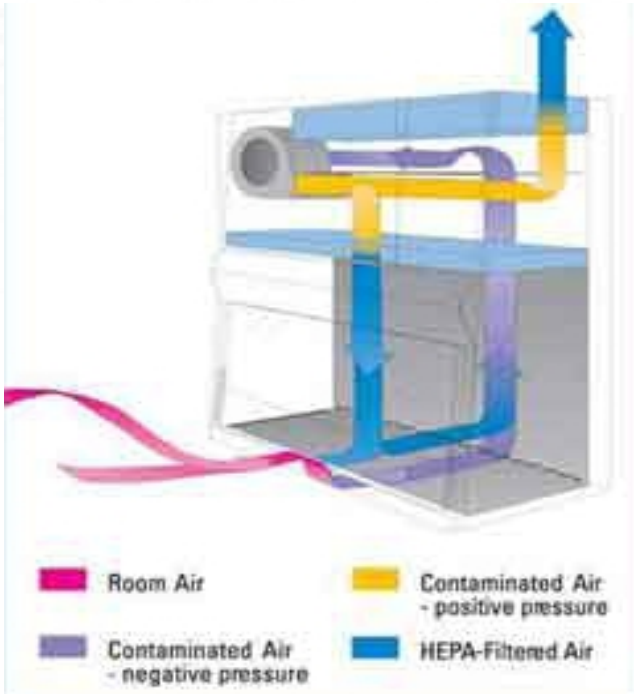
- Airflow patterns
  - Air is drawn into the cabinet through the front opening by the fan motor
  - Direction and velocity of airflow in the cabinet varies for different classes of cabinets

- 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

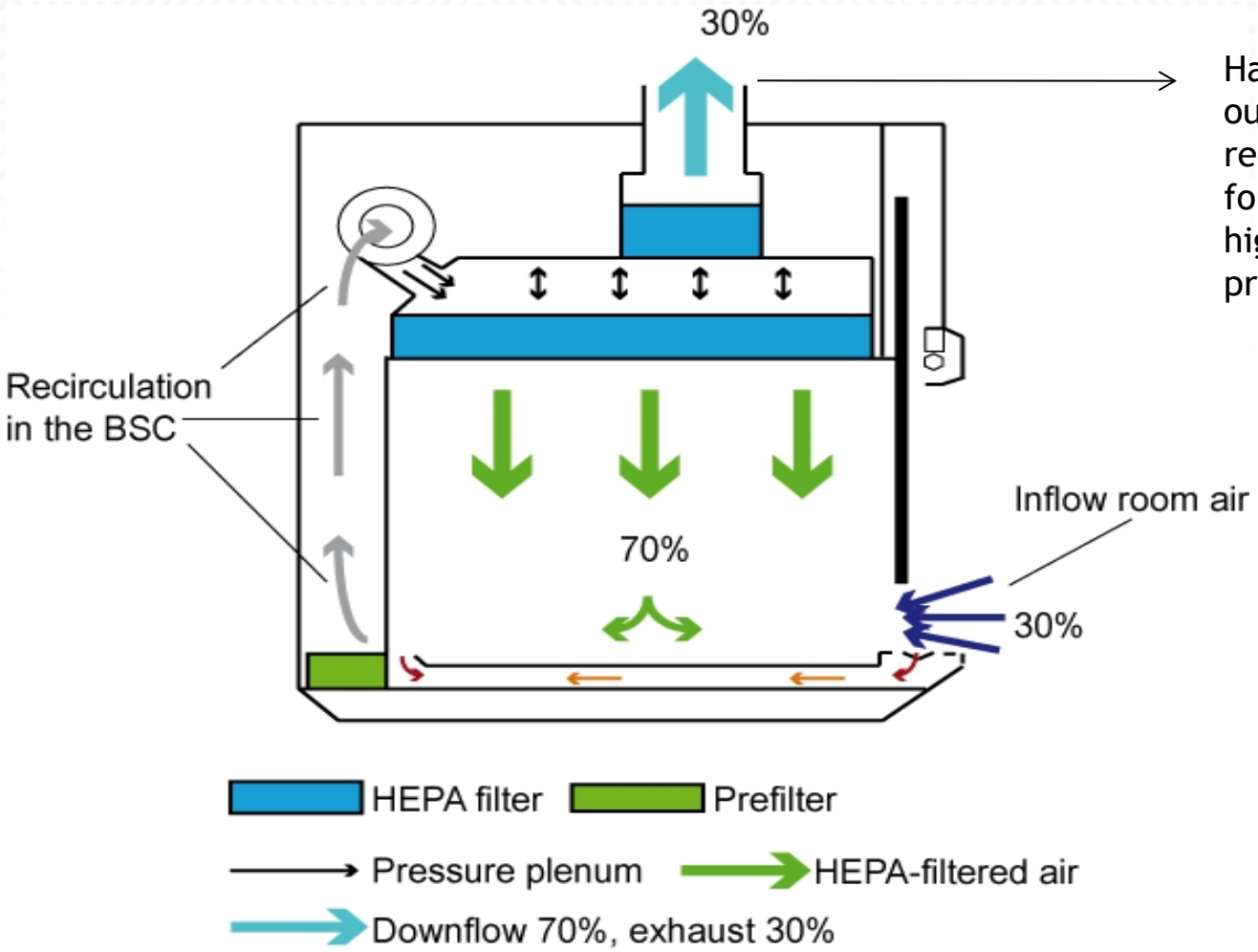
Type	Face velocity (lfpm)	Airflow pattern	TB precaution level	Product protection
Class I open front	75	In at front, rear and top through HEPA filter	Moderate, High	No
Class II Type A2	75	70% recirculated through HEPA; exhaust through HEPA	Moderate, High	Yes
Type B1	100	30% recirculated through HEPA; exhaust via HEPA and hard ducted	Moderate, High	Yes
Type B2	100	No recirculation. Total exhaust via HEPA and hard ducted	Moderate, High	Yes
Type B3	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		

Note: Laminar flow unit, fume hood or other stations are not to be used for manipulating a specimen or a culture

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
    - At installation,
    - Regularly at least once per year
    - Whenever BSC is moved
    - 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

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

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
    - Drastic increase in reading – filter clogged and needs to be changed
    - 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

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagonostics for a TB-Free Africa

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

Biosafety Cabinet cleaning Log

CTRL/BSC/ CWRU/006 **SERIAL # 6S-15-B-7619**
Model: BBF 6SS

Location: BSL3

MAINTENANCE:
Clean before and after with 5% Lysol/1% Bleach followed by 70% Ethanol

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				

Example of log sheet for recording daily maintenance for BSCs.

TB 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	

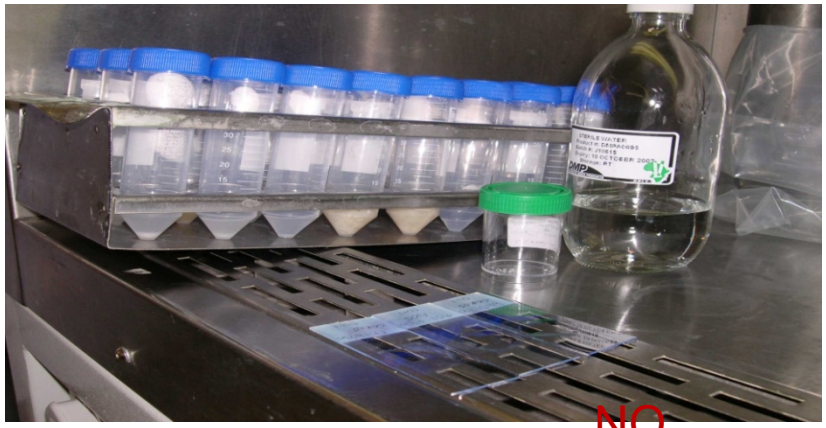
	Reagents and Media
1	Sodium Hydroxide (NaOH) solution 4%
2	Sodium Citrate solution
3	Phosphate Buffered Saline (PBS)
4	LJ
5	BD MGIT Tubes
6	PANTA
7	Tubes distilled water
8	NALC

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

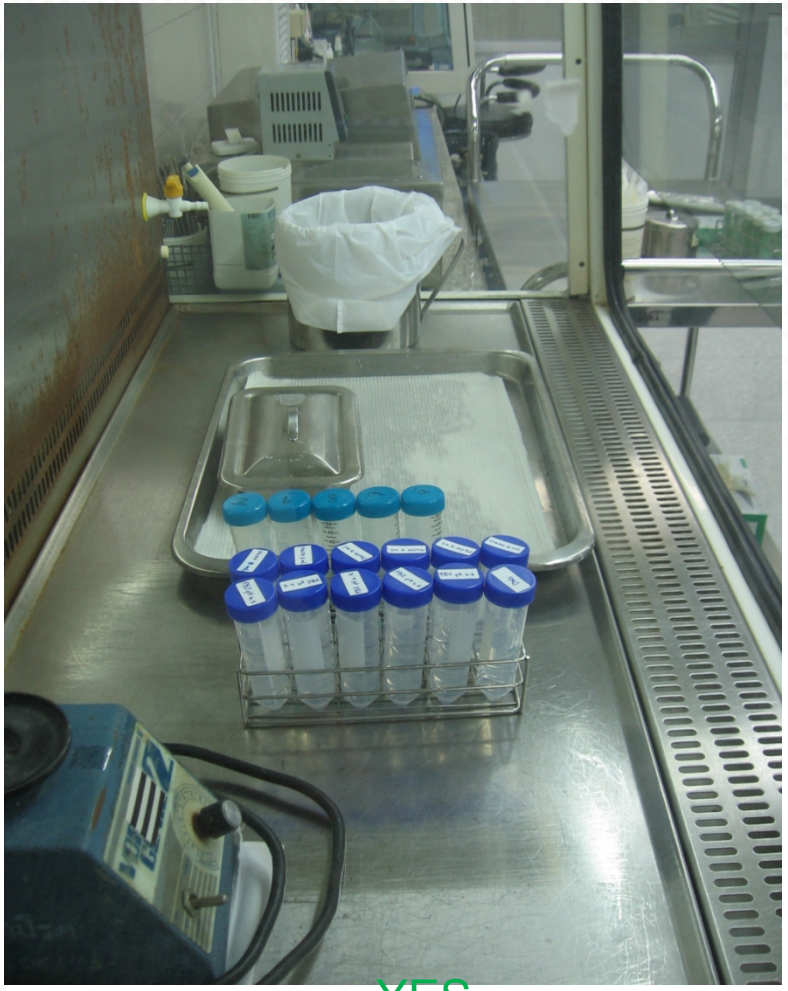
UGANDA Supranational® Reference Laboratory
Timely Accurate Diagonostics for a TB-Free Africa

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

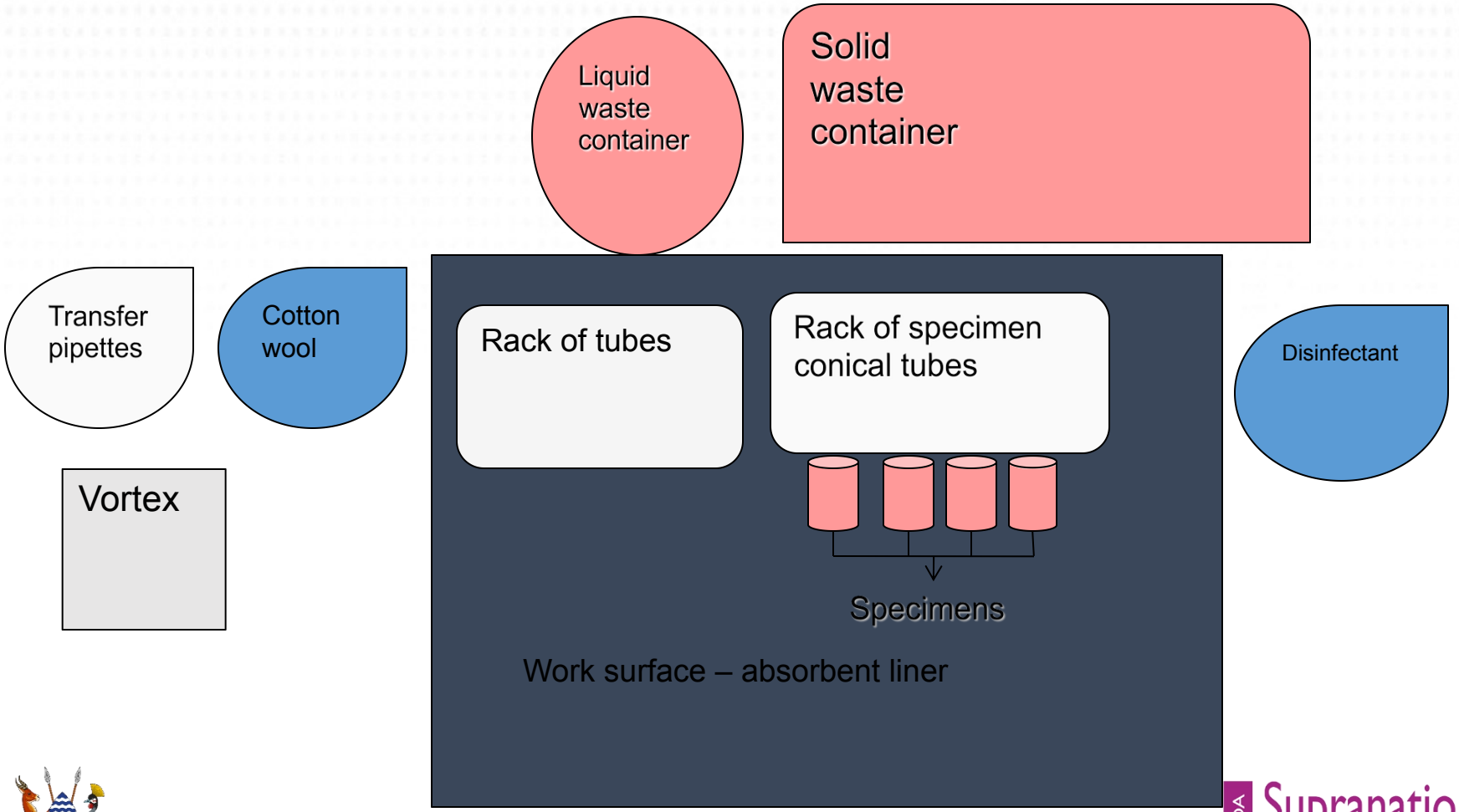


NO



YES

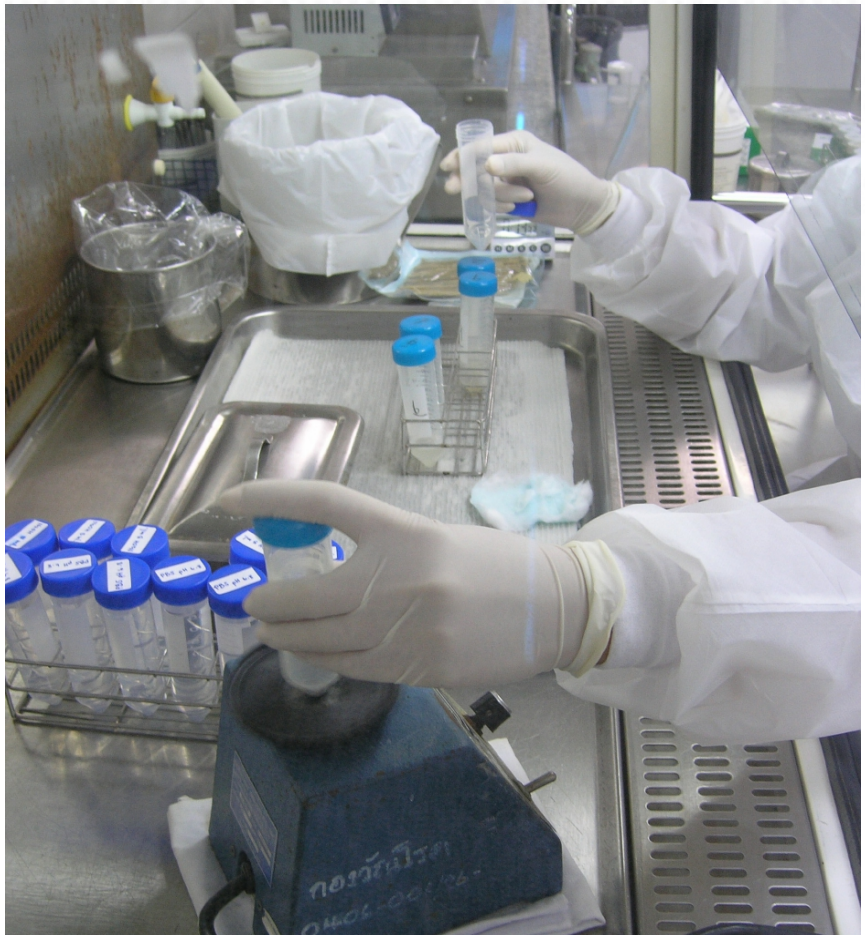
BSC placement



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

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

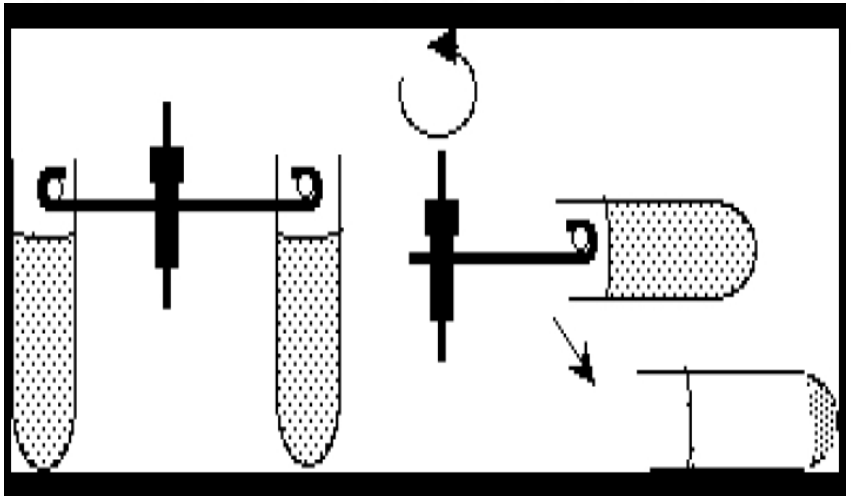
- Safety centrifuge cup
  - Air- and liquid-tight seal
  - Holds specimen tubes
  - Provides containment for aerosols that may be released during centrifugation

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

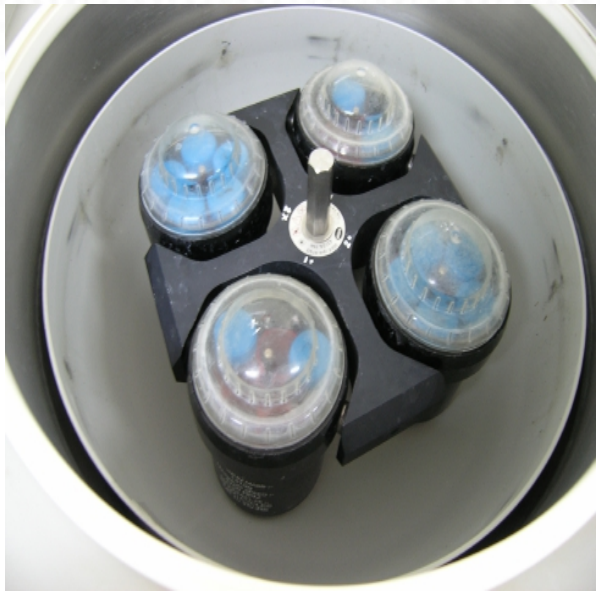
Swinging bucket rotors

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



Centrifuge recommendations for mycobacterial specimen processing

- Swinging bucket rotor
- Sealed centrifuge buckets (for safety!)
- Refrigeration (4 ^{o}C) to reduce the percentage of mycobacteria killed due to heat generated during centrifugation



- Required G force: <u>3000 x g (not RPM)</u>

Centrifuge: RPM vs RCF (g force)

- RPM = revolutions per minute
- RCF = relative centrifugal force or g force

These are not the same

- 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 x g required)

Calculating RPM required to reach desired RCF

Formula:

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

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

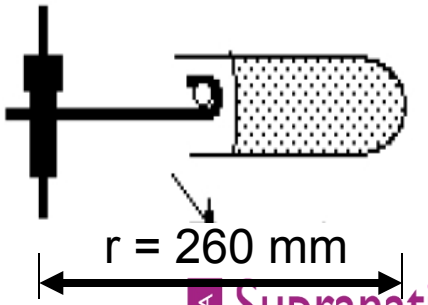
RCF = desired centrifugal force

Example: *r* = 260 mm *RCF* = 3000 x g

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

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

RPM = 3200



THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagonostics for a TB-Free Africa

Before use of the centrifuge:

- Inspect the interior of the bowl for stains
- Inspect rotors and buckets for cracks and signs of corrosion

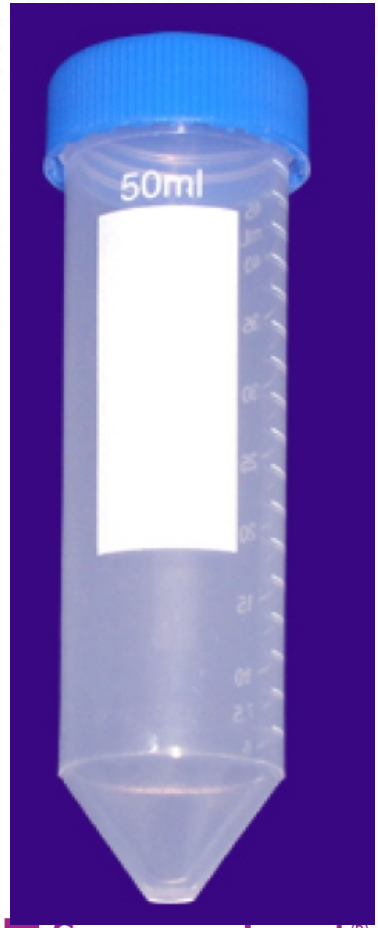
- Check log book to ensure centrifuge is in good working condition
- Pre-cool centrifuge prior to use upto 4°C

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



THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagonostics for a TB-Free Africa

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

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

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Incubators

- 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, Bangkok , Thailand

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

Summary points

- 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

ASSESSMENT

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

References

- Global Laboratory Initiative (GLI) http://www.gliquality.org/

nowledgement

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagnostics for a TB-Free Africa

DEPARTMENT OF HEALTH AND HUMAN SERVICES - USA

CDC

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagnostics for a TB-Free Africa

KOFIH
Korea Foundation for International Healthcare

gli
global laboratory initiative
advancing TB diagnosis

FIND
foundation
for innovative new diagnostics

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory
Timely Accurate Diagonostics for a TB-Free Africa