THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

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

Training on New and rapid Tuberculosis diagnostics (first and second line Probe Assay)

Module 6: Use and maintenance of LPA equipment

Uganda Supranational Reference Laboratory

Outline

- Identification of the various equipment used in LPA
- Purpose of the various LPA equipment
- Maintenance of the LPA equipment
- Precautions taken when using various equipment or when working in the different LPA rooms.

Exercise

1. In your groups identify at least 8 equipment used in the LPA laboratory

2. Name the purpose for each of the equipment named above

3. What are the maintenance procedures for the above mentioned equipment?

Genotype MTBDRplus equipment

1. Centrifuge
2. Vortex
3. Water bath
4. Heating block
5. Dry oven
6. Sonicator
7. PCR hood
8. Thermocycler
9. Twincubator
10. GT-Blot

Centrifuge

Sedimentation by centripetal force

- RPM (revolutions per minute) or x g
- RPM = the number of full rotations completed in one minute
- x g = centrifugal force
- Different sizes: microcentrifuge/picofuge (0.2 ml – 2.0 ml)

Non-Refridgerated micro centrifuge

Refridgerated micro centrifuge

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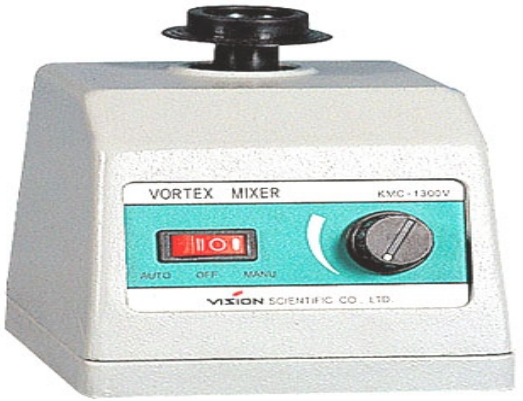
Centrifuge

!!!!! Important:

- Wipe with a clean cloth after use
- Record use on the log sheet provided by the individual laboratories
- Record maintenance activities

Vortex

- Distribute contents uniformly
- (mix reagents)



Vortex

Water bath

- Temperature stability for liquids
- It is crucial to heat samples in order to totally lyse cells and inactivate vegetative bacteria

Water bath

Vortex and water bath

!!!! Important

- The vortex must be disinfected on a regular basis
- Water in the waterbath must be replaced at regular intervals (e.g . weekly/ monthly)
- Clean with mild detergent
- Keep the heating element covered

Heating block

- Temperature stable
- Heat several tubes at the same time

Important:

- Clean the heating block with 70% ethanol after use

Heating block

Dry oven

- May use instead of heating block and waterbath
- External thermometer to record temperature
- Place specimen racks inside oven and do not remove once temperature is stable
- Transfer specimen tubes to heated racks
- Clean as required with 0.5% bleach and 70% alcohol (turn off before cleaning)

Dry oven

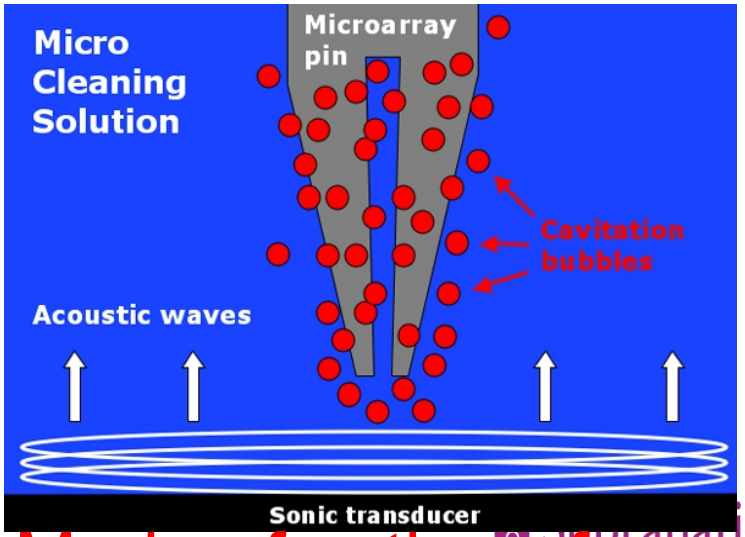
*80° C for 1 hour to kill tubercle bacilli

Sonicator

Ultrasound waves agitate particles and break bonds

Sonicator

Cavitation bubbles implode and generate powerful shockwaves that disrupt bacterial cell walls



Mode of action of a sonicator

Sonicator

!!! Important:

- Fill chamber with deionized water and de-gas to remove bubbles
- Do not place items directly on the bottom of the unit
- Make sure tubes are submerged in the water and the lids are floating on top

Maintenance:

- Weekly:
  - Drain water out of chamber
  - Clean inside and outside chamber with a soft cloth
  - Do not touch coils on bottom
  - Fill with deionized water

PCR hood

- Used in the 'clean room'/DNA-free room
- For preparation of the "master mix"
- NO DNA in this room
- Prevent contamination

PCR hood

PCR hood

!!!! Important:

- Clean before and after with bleach (prepared daily)
- Switch on the UV light after use
- Make sure the UV light is switched off while working in the PCR hood
- Allocate equipment for the “clean room” and DO NOT USE IN OTHER ROOMS
- Do not enter the clean room after DNA extraction/entering of amplification room

Thermocycler

- Designed for the amplification of the specific gene target by polymerase chain reaction
- High accuracy – amplification profiles can be modified to optimize results

!!! Important

- Switch off and unplug before cleaning
- Clean outside housing with ethanol and wipe clean after use

Thermocycler

TwinCubator

- Designed for use with DNA strips in reverse hybridization assays
- Manual procedure
- Developed for the Hain molecular diagnostic assays
- Dry incubation (no water bath needed)
- ONLY 12 SAMPLES

Twin incubator

Twincubator

!!! Important:

- Clean before and after use
- Switch off and unplug before cleaning
- Clean outside housing with 70% ethanol, wipe clean

Technician performing the LPA procedure

GT-Blot

- GT-Blot 20 and GT-Blot 48
- The GT-Blot is a fully automated system for hybridization
- Optimization of assay (prc

GT-blot 20

GT-blot 48

GT-Blot

!!!! Important:

- Wash machine before and after use
- Remove reagents after the last run of the day – wash, take “connectors” out of water at the back
- Do not leave the connectors in water
- Clean outside housing with ethanol
- Wash trays with water after each run
- Wash trays (not the plastic trays) with bleach once a week and do not leave the tray in the bleach

Scanner

The strips can be scanned and interpreted in less than 1 minute

Genoscan

Assessment

1) List at least 5 key LPA equipment.
2) Identify one way of maintaining each of the named 5 equipment.
3) What are the advantages and disadvantages of using a GT Blot compared to the twincubator?

Summary

- Maintenence logs should be in place for all the LPA equipment.
- A service contract should be in place for all the LPA equipment.
- Good laboratory practices should me maintained in the LPA lab despite dealing with non viable organisms.

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- www.hain-lifesciences.com

Acknowledgments

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