THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on Proficiency Testing Scheme GeneXpert DTS

Module 5: Equipment, supplies and reagents in preparation of DTS panels

Venue

Presenter

Date

Introduction

Genexpert DTS preparation is designed to be a simple, safe, stable and cost effective procedure using equipment and supplies typically found in most of the TB culture labs.

Objectives

By the end of this module participants should be able to

- To know all the equipment needed in the preparation of genexpert PT panels
- Describe the principle ,purpose use and maintenance of essential equipment
- To know all the supplies and reagents needed in the preparation of genexpert PT panels

Module outline

- General rules for equipment
- Essential equipment
- supplies and reagents

General Rules for All Equipment

- SOPs developed
  - Based on the operators manual
  - Read and reviewed annually by staff
- Staff trained to use and maintain equipment
  - Documented training in file
- The operators manuals should be available
  - Read and reviewed annually by staff
- A maintenance schedule as required by the manufacturer should be established
- Any corrective action or repairs should be documented

Equipment, Supplies and Reagents

Equipment

- GeneXpert Dx System
- Class II Biosafety Cabinet
- MGIT instrument
- Hot Air Oven
- Vortex
- Incubator 35-37 C
- Freezer

Supplies and reagents

- PPE, Timers
- Bio-transport carrier
- Trolley cart
- printer
- Repeat pipette
- Automatic pipettes P10-P1000
- Serological pipette aid
- Beakers
- Racks for tubes

GeneXpert Dx System

Purpose

Used for testing PT stocks for pretest and validation

Components

- Equipped with 6-color modules and GX2.1 software or higher
- GeneXpert instrument
- Computer
- Barcode wand reader
- Printer

GeneXpert Dx System Maintenance

Daily

- Remove used cartridges

Weekly

- Restart computer

Monthly

- Back up and archive database
- Disinfect Xpert
  - Exterior Xpert surface
  - Cartridge bay interior
  - Syringe plunger rod

- **Annual**
  - Calibrate the modules annually, after 2000 tests/module

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Class II Biosafety Cabinet (BSC)

Purpose

Primary containment of biological hazards.

Components

- BSC is a ventilated, enclosed cabinet
  - Used during work on hazardous micro-organisms
  - Protects both operator and environment from exposure to infectious aerosols
  - Some classes of BSC protect materials in use from cross-contamination

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Features of a BSC

- **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 fibers
- Pleated to increase surface area and affixed to a frame
- 99.97% effective in removing particles as small as 0.3µm 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

Class II Biosafety Cabinet (BSC) Maintenance

- **Weekly**
  - Smoke test
- **Monthly**
  - Remove work tray
  - Clean and disinfect all surface and pan
  - Re-assemble BSC
- **Annually**
  - Full inspection, servicing and recertification by BSC engineers
  - Recertification Post in any changes to magnahelic reference ranges to daily log sheets
  - Retain Certificate of Performance

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MGIT MACHINE

Purpose

- Used for culturing and multiplying MTB
- Inactivation verification

Principal

- MGIT contains modified Middle-brook 7H9 broth base. When supplemented with MGIT Growth Supplement and PANTA, it provides an optimum medium for growth of a majority of mycobacterial species
    - Growth Supplement-for growth of MTB complex.
    - MGIT PANTA -to suppress contamination.

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BD Bactec MGIT 960 Instrument

- **Daily**
  - Test front panel lights
  - Check MGIT printer paper and replace if low
  - Test green and red LED lights in each drawer
- **Monthly**
  - Remove air filters and rinse
  - Replace with clean dry filters
- **Periodic**
  - Replace calibrator tubes prior to expiration

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MGIT Machine maintenance

- Do not use any tube that is
- cracked/defects.
- Do not use a tube if the medium is discoloured or cloudy

Hot Air Oven with Thermometer

- **Purpose**

Inactivation of MTB/NTM

- **Monthly**
  - Turn off oven and unplug
    - Wipe interior and exterior with disinfectant
    - Inspect cord and plug for frayed wires
- **Annually**
  - Verify temperature of thermometer against reference thermometer

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Incubators

Purpose

For incubating cultures at suitable conditions needed for TB growth

Maintenance

- Record temperatures daily using a separate thermometer that is calibrated
- Clean with disinfectant immediately after any infectious spills
- Clean thoroughly with disinfectant o a monthly basis

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

Vortexer

Purpose .

For homogenization of solutions

Maintenance

- Switch off when not in use
- Daily maintenance : cleaning before and after use with a disinfectant
- Annually serviced

Refrigerators/Freezers

Purpose .

Storage of reagents and DNA

Maintenance

- Should maintain the doors closed all the time
- Regularly maintain the temperatures to ensure stability
- Clean and defrost the freezers regularly

Repeater andAutomatic pipettes

Purpose

- For aliquoting and suspension of liquids

Mode of operation (principle)

Based on sanction pressure

Maintenance

- Kept in Upright position after use
- Calibrated when due
- Leaned with a disinfectant before and after use.

Supplies for DTS Preparation

- 4-ml cryovials with external thread caps
- 2-ml Nalgene cryovials with external thread caps
- 2-ml microcentrifuge tubes, sterile, skirted, screw-cap with O-ring
- Cryo-Babies labels 1.5
- 3-ml graduated sterile transfer pipettes
- Extended length filtered 100-200 µl pipette tips

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Supplies for DTS Preparation

- 3 mm glass beads, sterile
- 16 x 100 mm glass tubes with screw-cap, sterile
- 50-ml plastic conical tubes, sterile
- Disposable 10 µl loop, sterile
- Pipette tips, 20 µl 200 µl, 1000 µl sterile and filtered
- Repeat pipette syringe tips, 5ml or 10 ml, sterile, individually wrapped
- 10-ml , 25 ml serological pipettes

Media and Reagents

- MTB/RIF/Ultra assay kit (Cepheid)
- Sterile phosphate buffer pH 6.8
- Food coloring
- Bactec MGIT 7 ml tubes
- Bactec MGIT 960 supplement
- Middlebrook 7H11 agar plates
- Middlebrook 7H9 broth
- Glycerol

ASSESSMENT

1. List all the important equipment required to prepare genexpert PT panels.
2. List atlest five general rules of use and maintenance of equipment.

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 and kept for each instrument

REFERENCES

- ISO 13528:2005, *Statistical methods for use in proficiency testing by interlaboratory comparisons*
- ISO Guide 34, *General requirements for the competence of reference material producers*
- ISO Guide 35, *Reference materials — General and statistical principles for certification*
- Guide 34, ISO Guide 35 and ISO 13528 (homogeneity and stability)
- ISO/IEC Guide 98-3, *Uncertainty of measurement — Part 3: Guide to the expression of uncertainty in measurement* (GUM:1995)
- ISO/IEC 17011:2004, *Conformity assessment — General requirements for accreditation bodies accrediting conformity assessment bodies*
- ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

Acknowledgments