THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on *Mycobacterium tuberculosis* drug susceptibility testing (first and second line LJ DST)

Module 6: Preparation of LJ DST drug stock solutions

(First and second line drugs)

Venue:

Presenter:

Date:

Introduction and Objective

Introduction

•This module describes the processes involved in the preparation of LJ DST drug stock solutions and their subsequent management.

Objective

•At the end of the module, participants are expected to;

- Be able to prepare drug stock solutions to be used in the routine LJ DST technique
- Carry out QC for the prepared drug stock solutions

Module Outline

- Introduction on drug preparation
- WHO recommended drugs to be used for routine LJ DST technique
- Materials required for LJ DST drug preparation.
- Drug potency and purity
- Methodology of preparing drugs
- QC for the prepared drug stock solutions

Introduction on drug preparation

- All drugs tested for routine DST in the laboratory should be those listed in the WHO guidelines for the management of tuberculosis.

- Drugs used for susceptibility testing should never be from medicine used for treatment but only pure compounds from reputable manufacturers.

Introduction to drug preparation

- Drug stock solutions keep better when more concentrated, For any drug, a stock solution of 100mg in 10 ml should be prepared to make stock concentration of 10mg/ml.

- This should be done taking into consideration the potency of the individual drugs.

WHO RECOMMENDED ANTI-TB DRUGS

- ***FIRST LINE DRUGS;***
  - *Rifampicin*
  - *Pyrazinamide*
  - *Isoniazid*
  - *Ethambutol*

	2nd Line MDR TB regimen (2018)
GROUP A	Levofloxacin Moxifloxacin Bedaquiline Linezolid
GROUP B	Cyclocerine Clofazimine Terizidone Terizidone
GROUP C	Ethambutol Delamanid Amikacin (Streptomycin) Imipenem-cilastatin Meropenem Ethionamide Prothionamide *Para*-aminosalicylic acid

Materials/Equipment

- Balance sensitive to +/- 0.001
- Cryovials
- Diluents (0.4% NAOH,distilled water,pure methanol,dimethyl sulfoxide/DMSO
- Automatic pipette
- Spatula
- 28 mls universal bottles
- Freezer
- fridge

Materials/Equipment

- Pipette tips
- Complete 1st and 2nd line drug sets:

Isoniazid, rifampicin,ethambutol

Moxifloxacin, Levofloxacin, Amikacin

Potency/Purity

- Drugs are purchased as powders that vary in activity from lot to lot
- The activity of a specific drug lot is listed as potency or purity on the Certificate of Analysis (C of A) supplied by the vendor
- Drugs at or near 100% potency may be weighed out without adjustment (it can be assumed that 10 mg powder = 10 mg active drug)

Drug Certificate of Analysis

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Ofloxacin
Product Number	O8757
Product Brand	SIGMA
CAS Number	82419-36-1
Molecular Formula	$C_{18}H_{20}FN_3O_4$
Molecular Weight	361.37

TEST	SPECIFICATION	LOT 040M1313V RESULTS
Appearance (Form)	Powder	Powder
Solubility (Color)	Faint Yellow to Yellow-Green	Yellow-Green
Solubility (Turbidity)	Clear to Slightly Hazy 50 mg/mL, 1 N NaOH	Clear
Proton NMR spectrum	Conforms to Structure	Conforms
Carbon	59.0 - 60.6 %	59.9 %
Nitrogen	11.3 - 11.9 %	11.7 %
Purity (HPLC)	≥99 %	100 %
Recommended Retest Period	---------- 3 years	----------
Specification Date:		MAY 2010
Date of QC Release:		MAY 2010
Recommended Retest Date:		APR 2013
Print Date:		MAY 17 2010

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Potency/Purity

- Potencies may not be close to 100% because:
  - There are other materials in the powder, such as impurities, preservatives or solubilizes
  - Potency standards may be decades old and drug manufacturing processes have changed since the standards were established

Potency/Purity

- If the potency of a given drug is below 75%, it is advisable to adjust the amount of grams to be weighed in order to cater for the impurities

$$\text{Adjusted drug weight} = \frac{\text{Desired amount of drug}}{\text{Potency of the drug}}$$

Exercise

EXERCISE (5minutes)

The potency of Ethambutol dihydrochloride is 74.5%, How many grams of ethambutol dihydrochloride would you weigh to prepare 10ml of a 1% drug solution?

Drug preparation method

- Record on the LJ DST drug preparation log/work sheet.
- Store all the prepared drugs at -20 for 6 months.

Procedure

- Allow the drugs to warm to room temperature.
- Weigh the adjusted amount of grams of each individual drug into sterile properly labelled universal bottles.
- Dissolve in 10ml of the respective drug diluent to give a concentration of 10000ug/ml. Mix well and transfer 1.5ml of the individual drugs into the labelled sterile storage cryovials.

QC FOR prepared drug solutions

- Use certified control strains such as H37Rv (ATCC 27294) with already known results to control the LJ DST media prepared using the drug solutions.
- Document on the QC log.

NB;

All drugs in the lab should be stored at correct temperatures as per manufacturers recommendations.

Already thawed drug solutions should always be discarded after using them

ASSESMENT

- List some of the WHO recommended drugs for LJ DST setting
- List some of the materials required for LJ DST drug susceptibility testing ?
- Why do we adjust potencies for some drugs?
- For how long and at what temperature do you store LJ DST drug stock solutions?
- How do you quality control LJ DST drug stock solution?

Summary

- All drugs to be tested in any laboratory LJ DST programme should be those recommended by WHO and used in the routine treatment management in that country.
- Drugs that have a potency below 75%, the measured weight has to be adjusted to cater for the impurities such as impurities, solubilizers and preservatives.
- Quality control using standard control strains has to be done on LJ DST drug containing media before such media is used to run routine patient samples.

Summary

- Always store the prepared LJ DST drug powders according to manufacturers specifications.

- Stored drug stock solutions that are already thawed should always be discarded and not stored for future use,

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- First and Second Line drugs and Drug Resistance

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