THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

MGIT CULTURE

Module 12: Preparation of Ziehl-Neelsen (ZN) Reagents

Outline

- Materials and Equipment required for ZN staining techniques
- Reagents required for ZN staining techniques
- Procedures for preparing ZN staining reagents
- Quality Control (QC) of freshly prepared ZN staining reagents
- Storage of ZN staining reagents

Exercise (5mins)

1. Discuss the major reagents used for ZN staining technique as used in a mycobacterium.

Requirements for ZN Staining Techniques

- Reagents/Chemicals:
  - Basic Fuchsin powder
  - Phenol
  - Absolute Alcohol
  - Methylene Blue powder
  - Concentrated Sulfuric Acid
  - Purified water

- Equipment:
  - Weighing Balance
  - Measuring cylinders
  - Stirring plate
  - Magnetic stirrers
  - Heating block

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Requirements for ZN Staining Techniques

- Materials:
  - Flask
  - Funnel
  - Weigh paper
  - Clean reagents bottles
  - Labels
  - QC slides

1. PPE

Personal Protective Equipment: What must be worn when you work in the laboratory.

Eye Protection

Lab Coat

Long Pants

Closed Toed Shoes – no exposed skin around feet

Lab gloves – when required

2

Adapted from http://slideplayer.com/slide/5917018/

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Reagent Quality: Specifications

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Reagent Name	Specification
Basic fuchsin powder	Procured from reputable or certified manufacturers by the Biological Stain Commission
Phenol	Near colorless (any coloration indicate loss of quality)
Absolute Alcohol	Technical Grade..
Acid	Technical Grade..
Water	Distilled or purified recommended
Methylene Blue powder	Procure from reputable or certified manufacturers by the Biological Stain Commission

Adjusting for Dye Purity

- Decimal equivalent of 75% = 0.75
- 10 g / 0.75 = 3.99 g = 13.3 g
- The final weight of basic fuchsin powder must
- have 10 g of basic fuchsin available

Adjusting for Dye Purity

- Important when:
- 1. Preparing 1% carbol fuchsin
- 2. Basic fuchsin powder dye content below 85%
- Example:
- Basic fuchsin powder dye content = 75%
- Need 10 g basic fuchsin

Care and Use of weighing balance

1. Fragile, precise
2. Handle with care
3. Rest on leveled surface
4. Use SOPs or operating manual for trouble shooting in case problems, use, operation and maintance
5. Keep balance clean and dry

Using the weighing balance

- Zero weighing balance after placing weighing pan in position
- Add to powder into the weighing boat until the required weight is reached
- Transfer the powder to a container from which the stain will be prepared

± 0.1 g precision is sufficient

Water Quality

- The water for reagent preparation must be
- - Free of mycobacteria, and Flora
- - Distilled or purified water
  - Rain, tap or boiled waters are **not recommended** for reagent preparation

Preparation of 1% Carbol Fuchsin

- **Contains per liter:**
  - Basic fuchsin powder 10 grams
  - Phenol 50 grams
  - 95% ethanol or methanol 100 ml
  - Water up to 1 liter
- **Preparation:**
  1. Dissolve phenol in alcohol
  2. Dissolve the fuchsin completely in the mixture
  3. Add the water
  4. Mix well

Preparation of 0.3% Methylene Blue

- **Contains per liter:**
  - Methylene Blue powder 3 grams
  - Water 1 liter
- **Preparation:**
  1. Add 500ml of water to a 2-liter flask
  2. Add the methylene blue
  3. Mix until powder is completely dissolved
  4. Add remaining water, mix well

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25% Decolorising Solution

- **Contains per liter:**
  - Sulfuric acid (minimum 95%) 250 ml
  - Water 750 ml

- **Preparation:**

1. Measure 750ml of water into a 2-liter flask
2. Measure 250ml of concentrated Sulphuric acid in a cylinder
3. Pour it slowly into the flask containing the water, directing the flow of acid gently along the inner side of the flask
4. Stop and swirl flask regularly as a lot of heat is generated until all acid is added
5. Mix well and allow to cool before use

3% Decolorising Solution

- **Contains per liter:**
  - Hydrochloric acid (fuming) - 30 ml
  - 95% denatured alcohol - up to 970mls
- **Preparation:**
  1. Add one liter of 95% denatured absolute alcohol to a 2-liter flask
  2. Slowly add the acid
  3. Swirl to mix well
  4. Allow to cool before use

Quality controlling of newly prepared ZN staining reagents

- Use known positive and negative control slides
  - Positives should be: 1+ or exact counts
  - Negatives should be: known AFB negative specimen
- Positives control should to be stained once
  - Check for the number of AFB and staining quality
- Negatives control can be re used up to 3 times
  - Check for contaminating AFB
  - Ensure background blue and clean

Reagent, labeling and storage

- ZN reagents should be labeled with the following:
  - Batch number
  - Generic name and concentration of reagent
  - Date of preparation
  - Date of expiry
  - Preparer

- All ZN reagents should be stored in well stoppered, in a cool dark place away from direct sunlight

ASSESSMENT

- Why is quality control of ZN reagents is important?
- Why is accuracy in weighing necessary in reagent preparation?
- What is the safe method of diluting acid?
- Why are reagents stored in well stoppered containers, in a cool dark place away from direct sunlight?
- What is the standard form of labelling reagents?

Module summary

- Key materials and equipment required for ZN preparation
- Reagents used for ZN staining technic
- Procedures used to prepare ZN reagents
- Performing QC on ZN reagents
- Storage of ZN reagents

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- Laboratory Diagnosis of Tuberculosis by Sputum Microscopy | The Handbook | Global Edition
- TB AFB Smear Microscopy Trainer Notes

https://www.aphl.org/programs/infectious_disease/tuberculosis/TBCore/TB_AFB_Smear_Microscopy_TrainerNotes.pdf

Acknowledgments