

MODULE 5

Preparation of Ziehl-Neelsen Reagents

Learning Objectives

At the end of the module, participants will be able to

- Describe the importance of using quality chemicals for reagent preparation
- Prepare reagents required for the Ziehl-Neelsen method
- Describe the safety requirements for reagent preparation
- Use positive and negative control slides for the Quality Control of Ziehl-Neelsen reagents
- Explain the use and frequency of routine quality control procedures.

Content Overview

- **Equipment required for staining reagent preparation**
- **Reagents required for staining reagent preparation**
- **Methods for staining reagent preparation**
- **Quality control (QC) of freshly prepared staining reagents**
- **Storage of staining reagents**

Required Materials

- **Reagents and Chemicals:**

- Basic Fuschin
- Phenol
- Alcohol
- Methylene Blue
- Sulfuric Acid
- Purified water

- **Materials:**

- Flask
- Funnel
- Weigh paper
- Clean reagents bottles
- Labels

- **Equipment:**

- Weigh Balance
- Measuring cylinders
- Stirring plate
- Magnetic stirrers

- **Miscellaneous:**

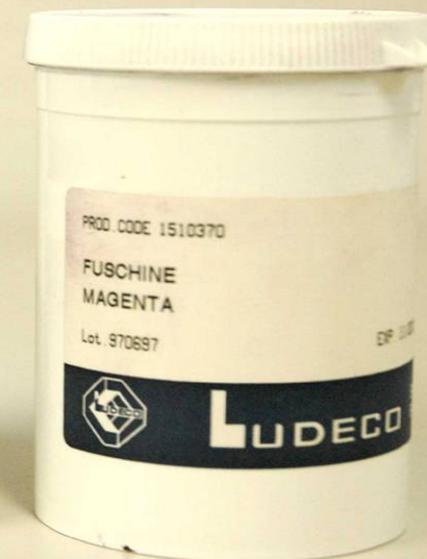
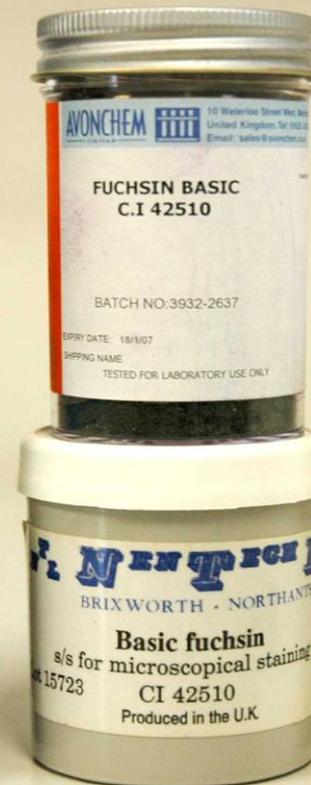
- Gloves
- Lab coat
- Safety glasses
- QC slides
- Register

Reagent Quality: Specifications

Reagent Name	Specification
Basic Fuchsin	Purchased from well known manufacturers or those certified by the Biological Stain Commission
Phenol crystals	Near colorless – never black
Alcohol	Technical grade
Acid	Technical grade
Water	distilled or purified, not tap water
Methylene blue	purchased from well known manufacturers

Dye Powder Specifications

FISHER SCIENTIFIC COMPANY Made in U.S.A.
Dyeing Manufacturing Division Fair Lawn, New Jersey 07410
M-297 771586 100 grams
Fisher Certified Biological Stain
7-11-78 **Methylene Blue**
Certified For Use in Histology
Bacteriology (Staining), Compounding
Blood Stains
C.I. No. 52015 Total Dye Content 86%
For laboratory and manufacturing use only, not for drug, food, or household use





Correcting for Dye Purity

- Only needed when

Preparing 0.3% carbol fuchsin AND basic fuchsin powder dye content below 85%

- Example:

Basic fuchsin powder dye content = 75%

Need 3 g basic fuchsin

Decimal equivalent of 75% = 0.75

$3 \text{ g} / 0.75 = 3.99 \text{ g} = 4.0 \text{ g}$

4.0 g of basic fuchsin powder must be weighed to have 3g of basic fuchsin available

Weighing Balance

- Fragile, precision instrument
- Handle with care
- Must be used on level surface
- Consult manual for operating instructions



Using the Balance

- High precision balance not needed: ± 0.1 g is sufficient
- Keep balance (and weights, if any) clean and dry
- Always use a weighing container or paper; correct back to zero

Water Quality

- Must be free of mycobacteria
- Use distilled or purified water
- NOT: rain water, tap water, or boiled water



0.3% Carbol Fuchsin

Use method recommended by the NTP

- **Contains per liter:**

- basic fuchsin powder 3 grams
- phenol 50 grams
- 95% ethanol or methanol 100 ml
- water up to 1 liter

- **Preparation:**

- dissolve phenol in alcohol
- dissolve the fuchsin completely in the mixture
- add the water, mix well



0.3% Methylene blue

Use method recommended by the NTP

- Contains per liter:
 - methylene blue powder 3 grams
 - water 1 liter
- Preparation:
 - add 500ml of water to a 2-liter flask
 - add the methylene blue and mix until completely dissolved
 - add remaining water, mix well



Decolorising Solution

Use method recommended by the NTP

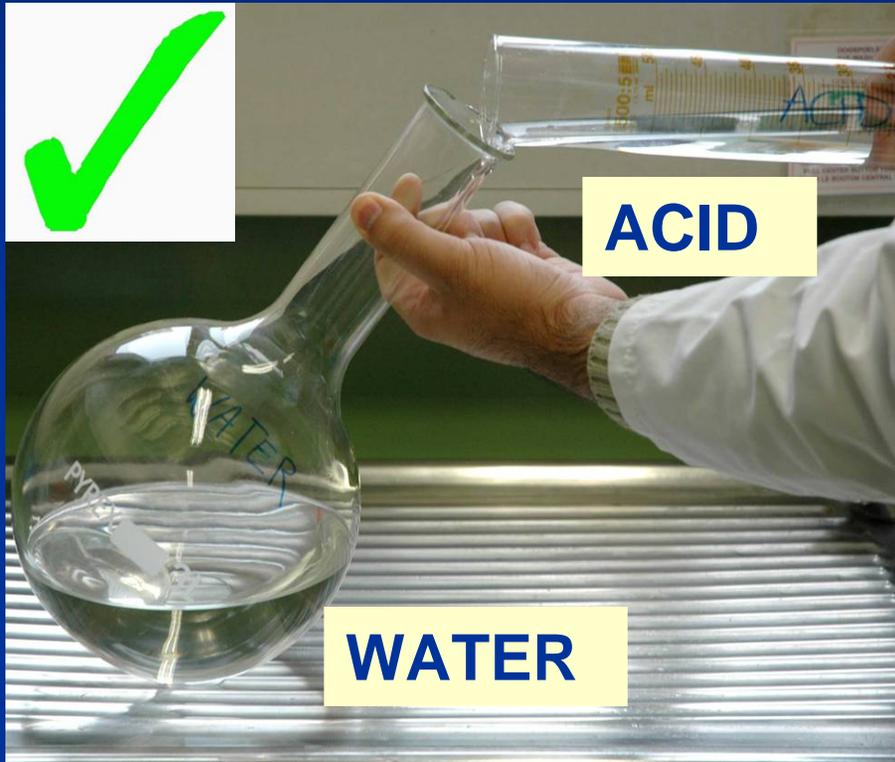
- **Contains per liter:**
 - sulfuric acid (minimum 95%) 250 ml
 - water 750 ml
- **Preparation:**
 1. Measure 750ml of water into a 2-liter flask
 2. Measure 250mL of concentrated sulphuric acid in a cylinder
 3. Pour it *slowly* into the flask containing the water, directing the flow of acid gently 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



Alternative Decolorising Solution

- Contains per liter:
 - Hydrochloric acid (fuming) - 30 ml
 - 95% denatured alcohol - up to 1 liter
- Preparation:
 - Add one liter of 95% denatured alcohol to a 2-liter flask
 - Slowly add the acid
 - Mix well and allow to cool before use

Diluting Acids Safely



Always add acid to water



NEVER add water to acid

Quality Control of New Staining Reagents

- Always needed; record results in logbook
- Positive as well as negative control slides
 - Positives: 1+ TB patient sputum; exact counts
 - Negatives: known AFB negative specimen
- Positives to be stained once
 - Check for the number of AFB and staining quality
- Negatives: ZN stain repeated 3 times
 - Check for contaminating AFB
 - Ensure background blue and clean

Reagent Labeling and Storage

- Each bottle or container must be labeled with at least
 - Name of reagent
 - Date of preparation
- Store all reagents in a cool place away from direct sunlight

Summary

- Why must quality reagents be used to prepare staining reagents?
- Why is accurate preparation of reagents necessary to obtain optimum staining results?
- What is the role of control smears in evaluating the performance of newly prepared staining solutions?
- Why should reagents be stored in clean bottles and out of direct sunlight?