

Differentiation of acid-fast microorganisms in smears & sections**Principle of method**

This kit contains all reagents required to carry out the traditional Ziehl-Neelsen staining procedure for the identification of acid-fast microorganisms in smears and sections.

Each Kit contains the following reagents:

ZN-Carbol Fuchsin	250ml
TB-Differentiator solution	2 x 250ml
Counterstain solution	250ml

Storage & stability

Keep the reagents in the kit packaging provided and ensure that the nozzles are closed to prevent evaporation & oxidation from the air. Store the kit at room temperature in a darkened cupboard. The kit reagents will remain stable in these conditions. This kit is for "In-vitro" diagnostic use only.

Preparation of reagents and usage information

In use carefully apply the nozzle to the slide and squeeze out sufficient reagent to cover the sample. Excessive pressure is not required to release the reagent, ensure that the opening in the nozzle is sufficient to allow the reagent to flow without excessive pressure being applied.

The reagents are now ready to use.

Use a known positive control with the test slide to confirm that the reagent system is working.

Information obtained from stained slides in concentration procedures should always be backed up by culturing techniques.

Method

Prepare a 3-4² cm thin smear with as little mechanical manipulation as possible. Excessive movement may cause damage to bacterial cells and loss of acid fastness. Heat fix by passing the slide, smear side up, through a bunsen flame 5-6 times.

1. Working in a fume hood (**Phenol fumes are released - TOXIC**). Cover smear with a piece of filter paper slightly smaller than the slide, flood with ZN-Carbol fuchsin solution, gently heat over bunsen flame or hot plate until steaming, do not allow to boil or dry out. Keep filter paper flooded with stain. Keep hot for 3-5 minutes, or stain in a suitable vessel for 5 minutes at 90DC or 30 minutes at 55DC for thick smears or sections.
2. After cooling rinse briefly in tap water for 5-10 seconds.
3. Decolourise by running the TB-Differentiator solution over the smear. The decolouration time will vary with the thickness of the smear, generally 5-30 seconds for thin smears, up to 2 minutes for thick smears and 5-10 minutes for sections until tissue is pink. After decolourisation rinse briefly in tap water.
4. Flood slides with counterstain for 1-2 minutes.
5. Rinse briefly in tap water and examine.

The method given in this leaflet is designed as a general guide only and should be modified by the laboratory to suit the samples being processed to obtain optimal staining and decolorisation times.

Results

Acid fast organisms	-	Bright red-pink
Non-acid fast organisms & background material	-	Blue/green

GCC Diagnostics guarantees that the highest quality reagents are supplied with this product to give reliable results within the lifetime of this kit stored as directed. The user should however, determine the suitability of this product for their intended use.

Unsatisfactory performance

As part of our duty to monitor product performance and our policy of continual improvement

Please report to us any unsatisfactory performance you may experience with this product. In the event of any reagent degrading before expiry of shelf life please contact us for a replacement

MSDS Section

Carbol fuchsin -ZN contains organic dyestuffs <1%, Alcohols <15% Glycols <15% Phenol <5%, balance water.

TB Differentiator contains mineral acid <3%, Alcohols <50%, balance water.

Methylene blue counterstain contains organic dyestuffs <2%, glycols <10%, balance water.

Malachite green counterstain contains organic dyestuffs <2%, glycols <10%, balance water.

When using this product observe the usual rules for the safe handling and use of laboratory reagents, wear suitable body, face and eyes protection. Ensure eye bath station is nearby. There is a potential danger of liquid reagent squirting out of the nozzle of the bottle by accident or in mis-use, be aware of this potential danger. Keep top on reagent bottles at all times when not in use.

ZN Carbol fuchsin - contains phenol which is TOXIC and can be absorbed through the skin and by inhalation and ingestion. H301+311+332 Toxic if swallowed, by skin contact and inhalation. H319 Causes serious eye irritation. P260 Do not breathe vapour. P262 Do not get in eyes, skin, clothing. P285 In case of inadequate ventilation, wear respiratory protection. P301 If swallowed, rinse mouth with water, seek emergency medical attention.

TB Differentiator - H224 Flammable irritant liquid and vapour. H302+H312+H333 Harmful if swallowed, by skin contact and inhalation. P233 Keep container tightly closed. P210 Keep away from sources of ignition. P262 Do not get in eyes, skin, clothing.

IF SWALLOWED - rinse mouth with water, seek urgent medical attention. IF INHALED - move to clear air zone. IF ON SKIN OR IN EYES - rinse with soap and water and apply eye batch. Seek medical attention if you feel unwell

Methylene blue/ Malachite green counterstain - H304+313 Harmful if swallowed, enters airways by skin contact.

Keep container tightly closed. P262 Do not get in eyes, skin, clothing.

IF SWALLOWED - rinse mouth with water, seek urgent medical attention. IF INHALED - move to clear air zone. IF ON SKIN OR IN EYES - rinse with soap and water and apply eye bath. P314 Get medical attention if you feel unwell.

Waste disposal of reagents

All reagents in this kit may be disposed of down the public sewer system by dilution with water, however check with local sewerage authority about the disposal of small amounts of phenolic residues.

The cleaned empty packaging should be recycled as polyethylene, cardboard, paper.

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