mZN Staining Protocol

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Abstract

The Modified Ziehl-Neelsen stain (mZN stain) is a type of differential bacteriological stain used to identify acid-fast organisms, mainly *Mycobacteria*. Acid fast organisms are those which are capable of retaining the primary stain when treated with an acid (*fast=holding capacity*). Members of the Actinomycetes, genus *Nocardia* (N. *brasiliensis* and N. *asteroides* are opportunistic pathogens) are partially acid-fast. Oocysts of coccidian parasites, such as *Cryptosporidium* and *Isospora*, are also acid-fast. Hence they can also be detected and identified through mZN staining procedure.

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Materials

- Carbol-Fuchsin by Contributed by users
- Distilled Water by Contributed by users
- Methanol M3641 by Sigma Aldrich
- Disposable Latex Gloves, Medium, 100/Box GL002M.SIZE.1PK by Bio Basic Inc.
- Methylene Blue M-680 by Gold Biotechnology
- Microscope slides by Contributed by users
- Compound Microscope by Contributed by users
- ethanol by BBI Biotech
- Acid Alcohol by Contributed by users

Protocol

Step 1.

Spread the stool sample evenly on the middle of the slide with constant rotational movement.

AMOUNT

3 mg Additional info:
(Amount of stool sample)

DURATION

00:10:00 Additional info: (5 to 10 minutes) for rotational movement

Step 2.

Place the slides on dryer with smeared surface upwards and air-dried it.

TEMPERATURE

60 °C Additional info:

DURATION

00:10:00 Additional info: minutes

Step 3.

Fix the dried smear with absolute methanol.

DURATION

00:05:00 Additional info: or (3-5 minutes)

Step 4.

Now, add the Carbol-fuchsine solution to the slide to cover the whole smear.

REAGENTS

✓ Carbol-Fuchsin by Contributed by users

DURATION

00:20:00 Additional info: minutes

Step 5.

Wash the slide gently with tap water with the help of a dropper.

SAFETY INFORMATION

Do not expose the slides to the high pressure of tap water directly, rather it will be better to use a dropper for washing the slides.

Step 6.

After washing the slide, add decolorizer (Acid Alcohol) to the smear and wash the slide again with tap water.

AMOUNT

3 ml Additional info: or 4-6 drops

REAGENTS

Acid Alcohol by Contributed by users

Step 7.

Now, add the counter stain (Methylene Blue) and wash the slide with clean water.

REAGENTS

Methylene Blue M-680 by Gold Biotechnology

DURATION

00:05:00 Additional info: minutes wait for methylene blue

Step 8.

Clean the back side of the slide with a tissue paper and put it in the draining rack to air dry.

DURATION

00:05:00 Additional info: minutes, wait for slide to dry

Step 9.

Than examine the smear with the help of a compound microscope with 40x and 100x (immersion oil lens) objective and scan the smear throughly for parasite identification.

EQUIPMENT

Equipment brand:
Olympus
SKU:
CH20i
Specifications:
Biological microscope , Anti-fungus treated optics , Built to last- Superior build quality

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