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Protocol status: Working
 We use this protocol and it's working

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Modified ZN Staining Protocol V.1

Forked from [mZN Staining Protocol](#)

PLOS One

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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.

MATERIALS

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- ☒ Carbol-Fuchsin **Contributed by users**
 - ☒ Distilled Water **Contributed by users**
 - ☒ Methanol **Sigma Aldrich Catalog #M3641**
 - ☒ Disposable Latex Gloves, Medium, 100/Box **Bio Basic Inc. Catalog #GL002M.SIZE.1PK**
 - ☒ Methylene Blue **Gold Biotechnology Catalog #M-680**
 - ☒ Microscope slides **Contributed by users**
 - ☒ Compound Microscope **Contributed by users**
 - ☒ ethanol **BBI Biotech**
 - ☒ Acid Alcohol **Contributed by users**
- #### STEP MATERIALS
- ☒ Carbol-Fuchsin **Contributed by users**
 - ☒ Acid Alcohol **Contributed by users**
 - ☒ Methylene Blue **Gold Biotechnology Catalog #M-680**
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 - ☒ Acid Alcohol **Contributed by users**
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1 The stool sample was Spread evenly on the middle of the slide with constant rotational movement.

⌚ 00:10:00 (5 to 10 minutes) for rotational movement

⚖ 3 mg (Amount of stool sample)

- 2 The slides were then placed on dryer with smeared surface upwards to air-dried them.

🔥 60 °C

⌚ 00:10:00 minutes

- 3 The dried smear was fixed with absolute methanol.

⌚ 00:05:00 or (3-5 minutes)

- 4 Now, the Carbol-fuchsin solution was added to the slide to cover the whole smear.

☒ Carbol-Fuchsin **Contributed by users**

🕒 00:20:00 minutes

- 5 The slides were washed 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.

- 6 After washing the slide, decolorizer (Acid Alcohol) was added to the smear and the slide washed again with tap water.

☒ Acid Alcohol **Contributed by users**

🧴 3 mL or 4-6 drops

- 7 Then the counter stain (Methylene Blue) was added and left for 5 minutes and then washed the slide with clean water.

☒ Methylene Blue **Gold Biotechnology Catalog #M-680**

🕒 00:05:00 minutes wait for methylene blue

- 8 The back side of the slides were cleaned with a tissue paper and put in the draining rack to air-dry.

🕒 00:05:00 minutes, wait for slide to dry

- 9 The smear was examined with the help of a compound microscope with 40x and 100x (immersion oil lens) objective and scanned thoroughly for

parasite identification.

Equipment	
	NAME
Olympus	BRAND
CH20i	SKU
Biological microscope , Anti-fungus treated optics , Built to last- Superior build quality	SPECIFICATIONS