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Modified ZN Staining Protocol V.2

 Version 1 is forked from [mZN Staining Protocol](#)

 [PLOS One](#)

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 15450



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

MATERIALS

- ☒ Carbol-Fuchsin
- ☒ Distilled Water
- ☒ Methanol **Merck MilliporeSigma (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
- ☒ Compound Microscope
- ☒ ethanol **BBI Biotech**
- ☒ Acid Alcohol

STEP MATERIALS

- ☒ Carbol-Fuchsin
- ☒ Acid Alcohol
- ☒ Methylene Blue **Gold Biotechnology Catalog #M-680**



Protocol materials

- ⊠ Acid Alcohol
- ⊠ Carbol-Fuchsin
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- ⊠ Acid Alcohol
- ⊠ Methylene Blue **Gold Biotechnology Catalog #M-680**

- 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

 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


 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.

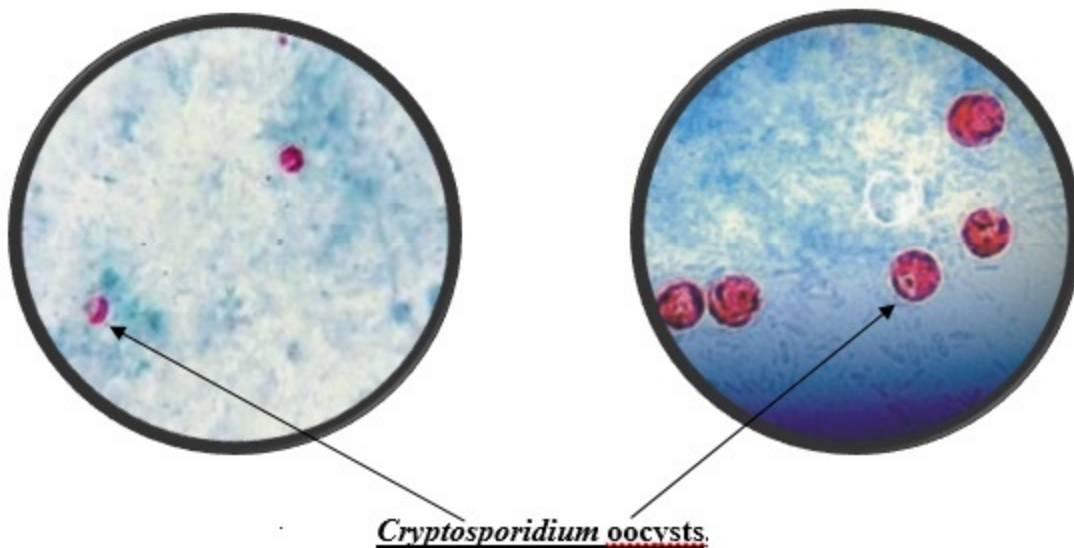
 Methylene Blue **Gold Biotechnology Catalog #M-680**

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