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Modified ZN Staining Protocol V.1

Forked from mZN Staining Protocol

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

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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

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

Protocol materials

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- Acid Alcohol
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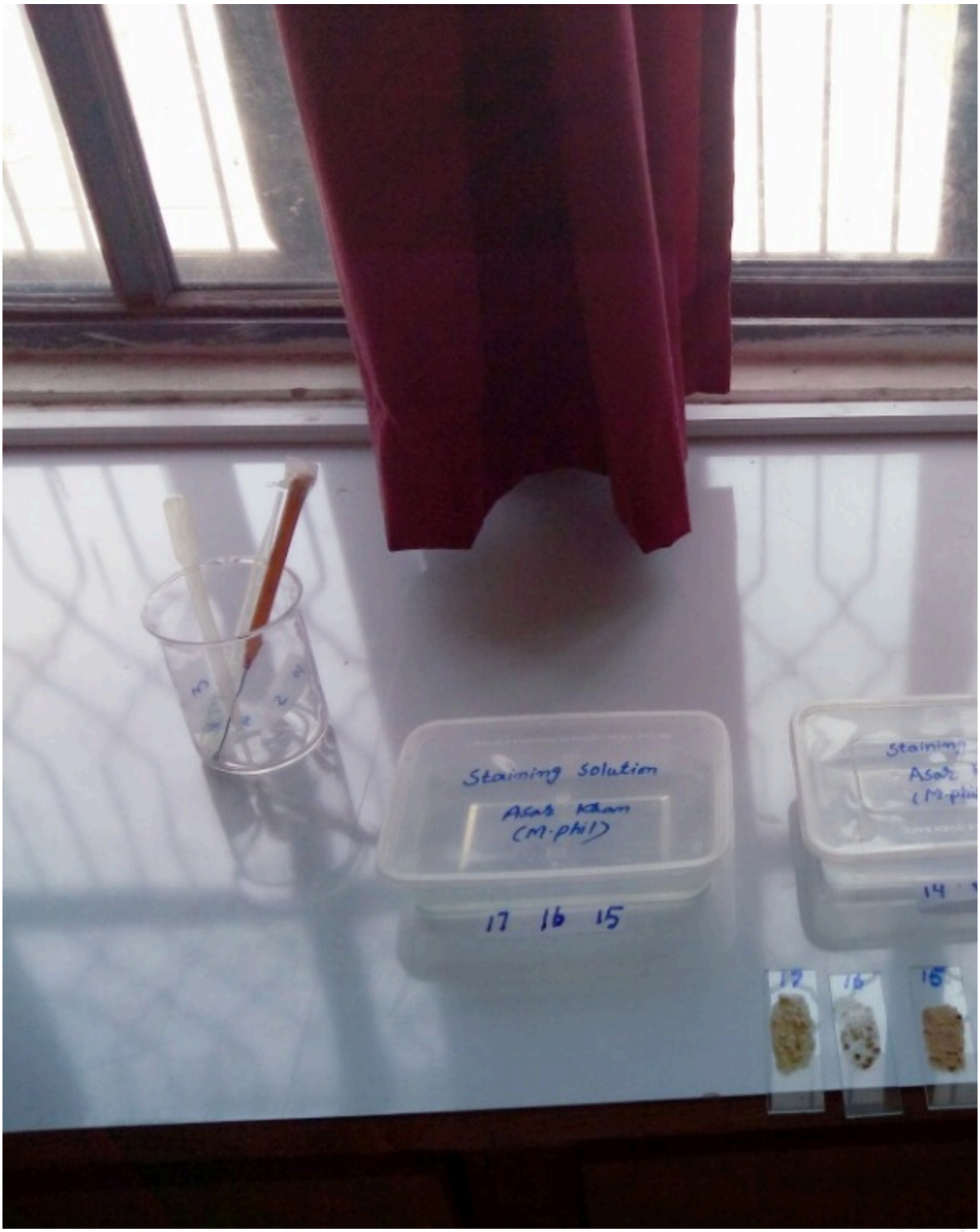
1 The stool sample was Spread evenly on the middle of the slide with constant rotational movement.



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

3 mg (Amount of stool sample)

2 The slides were than 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-fuchsine 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) twas added o 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.

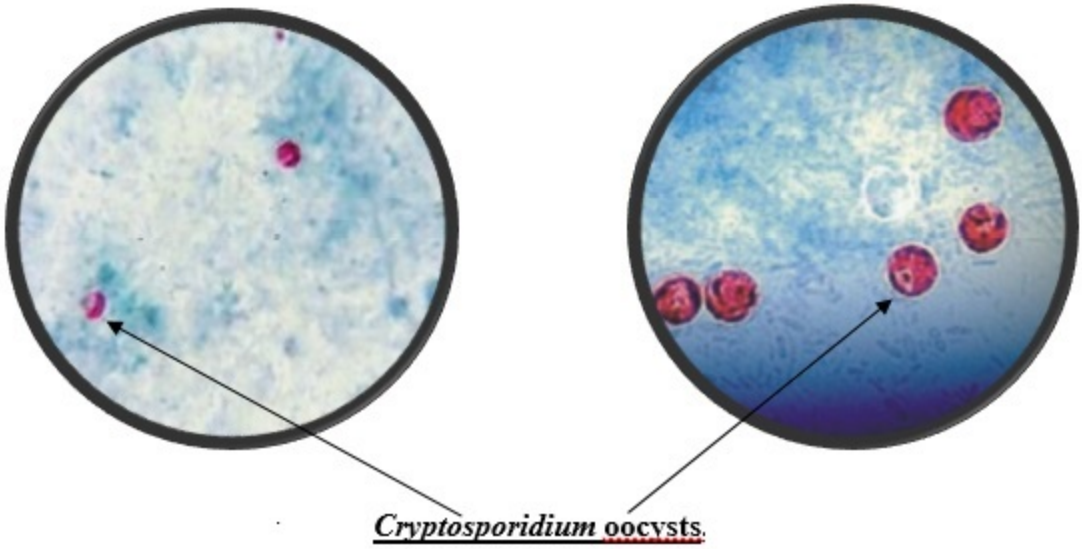
Methylene Blue **Gold Biotechnology Catalog #**M-680

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 throughly for parasite identification.



Cryptosporidium oocysts.

Equipment

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