Sep 07, 2018 Version 2

Modified ZN Staining Protocol V.2

Version 1 is forked from mZN Staining Protocol

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

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Keywords: mzn staining procedure, differential bacteriological stain, mzn stain, type of differential bacteriological stain, acid fast organism, mycobacteria, neelsen stain, primary stain, staining protocol, fast organism, cryptosporidium, actinomycete, oocysts of coccidian parasite, coccidian parasite, members of the actinomycete, genus, modified ziehl, opportunistic pathogen, modified zn

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

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- Carbol-Fuchsin
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- Carbol-Fuchsin
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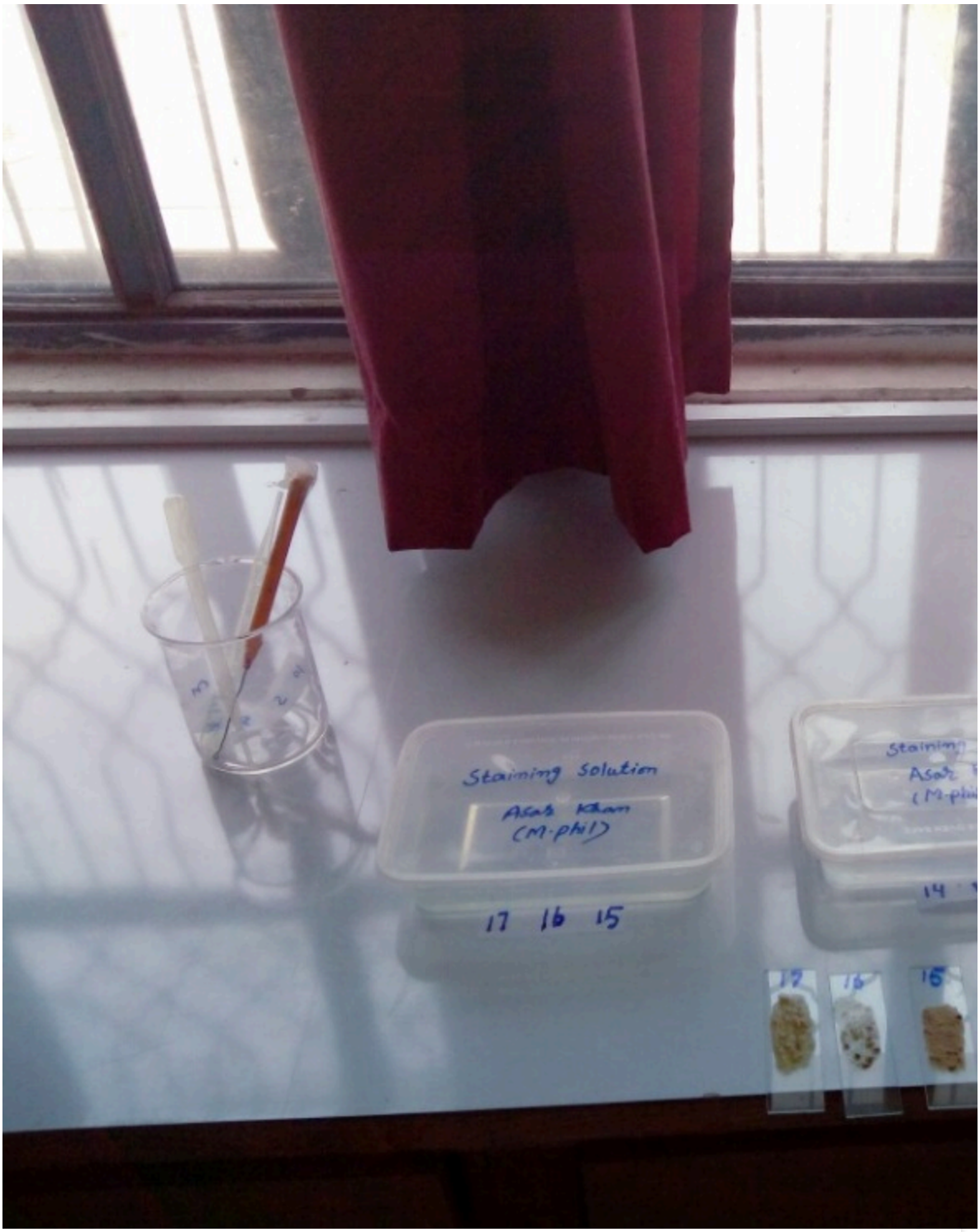
Troubleshooting

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.

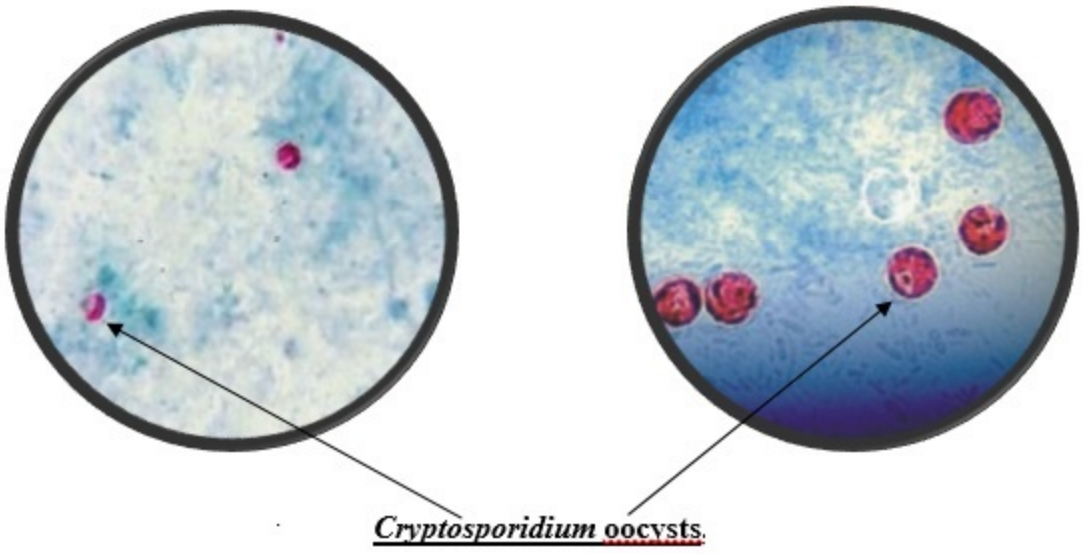
Methylene Blue **Gold Biotechnology Catalog #**M-680

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.



Equipment

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