Modified ZN Staining Protocol

Asar Khan,Sumaira Shams,Saima Khan,Muhammad Iftikhar Khan,Abid Ali

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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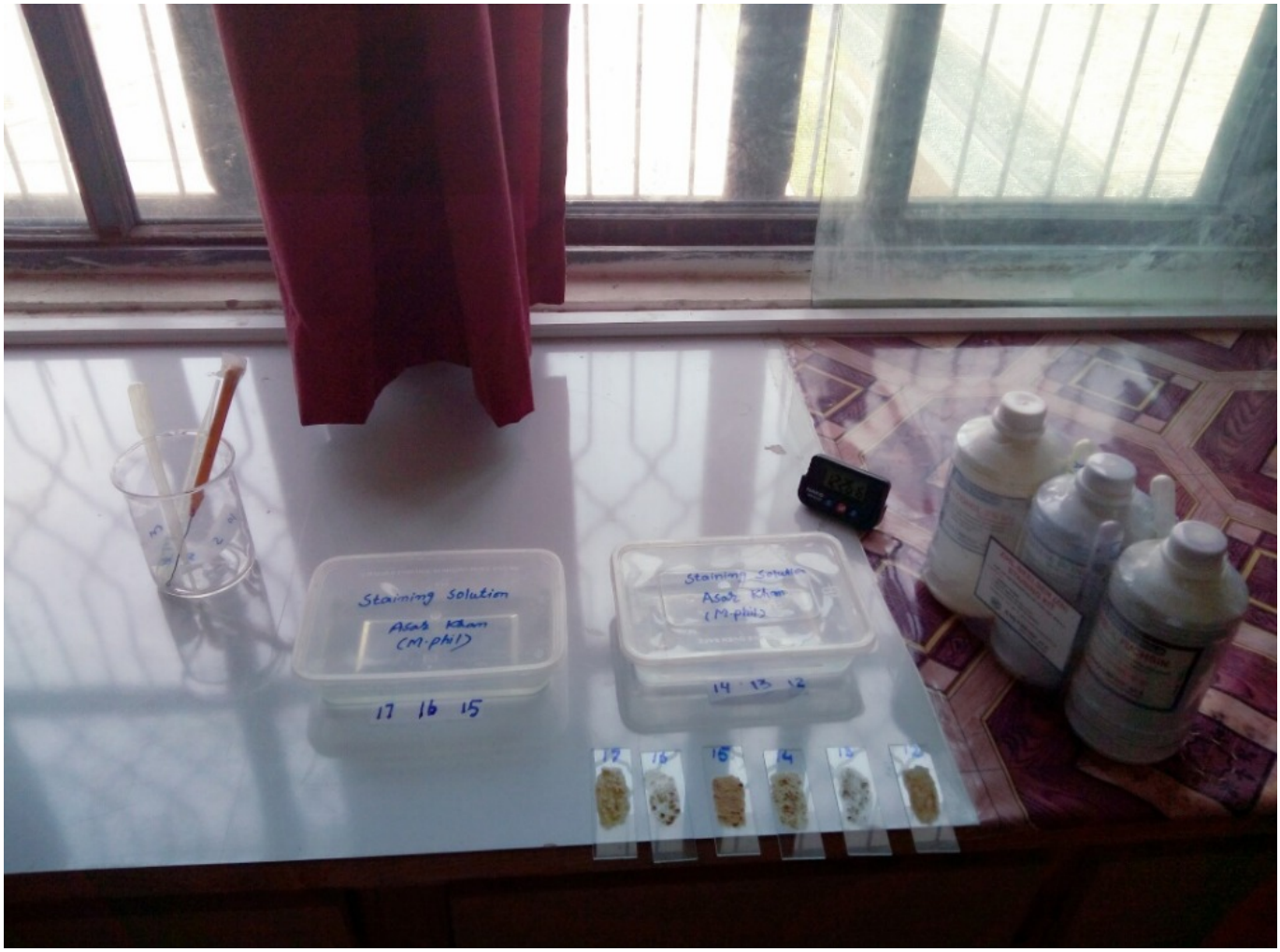
Materials

- 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.

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



AMOUNT
3 mg : (Amount of stool sample)
DURATION
00:10:00 : (5 to 10 minutes) for rotational movement

Step 2.

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

TEMPERATURE
60 °C :
DURATION
00:10:00 : minutes

Step 3.

The dried smear was fixed with absolute methanol.

DURATION
00:05:00 : or (3-5 minutes)

Step 4.

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

REAGENTS

Carbol-Fuchsin by Contributed by users

DURATION

00:20:00 : minutes

Step 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.

Step 6.

After washing the slide, decolorizer (Acid Alcohol) twas added o the smear and the slide washed again with tap water.

AMOUNT

3 ml : or 4-6 drops

REAGENTS

Acid Alcohol by Contributed by users

Step 7.

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

REAGENTS

Methylene Blue M-680 by Gold Biotechnology

DURATION

00:05:00 : minutes wait for methylene blue

Step 8.

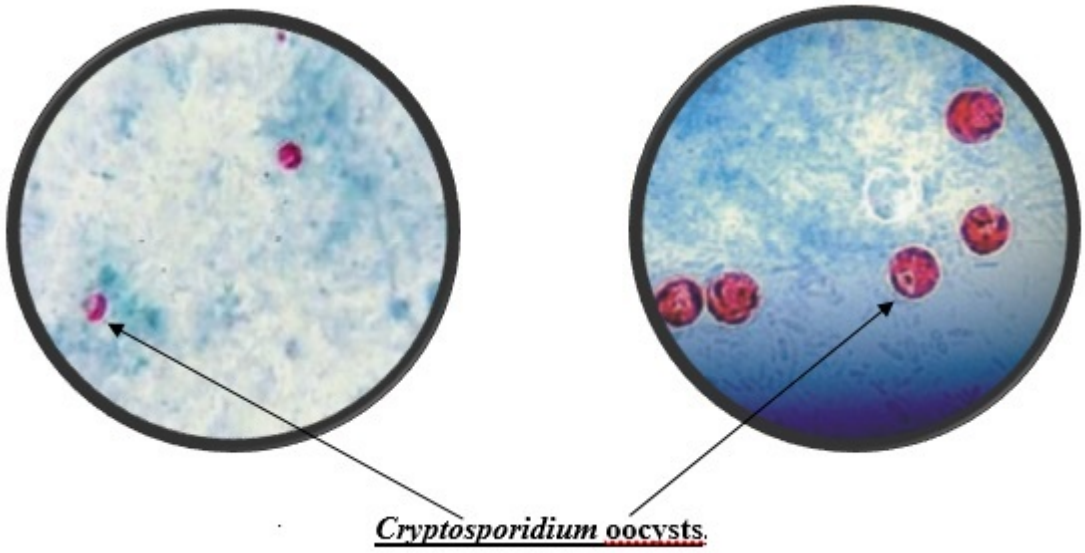
The back side of the slides were cleaned with a tissue paper and put in the draining rack to air-dry.

DURATION

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

Step 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

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

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