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

- **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**
- **Carbol-Fuchsin Contributed by users**
- **Acid Alcohol Contributed by users**
- **Methylene Blue Gold Biotechnology Catalog #M-680**
1. The stool sample was spread evenly on the middle of the slide with constant rotational movement.

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

3. The dried smear was fixed with absolute methanol.

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

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

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

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

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.