Sep 07, 2018 Version 2

## O Modified ZN Staining Protocol V.2

Version 1 is forked from <u>mZN Staining Protocol</u>

PLOS One

DOI

#### dx.doi.org/10.17504/protocols.io.tb2eiqe

## Asar Khan<sup>1</sup>, Sumaira Shams<sup>1</sup>, Saima Khan<sup>1</sup>, Muhammad Iftikhar Khan<sup>2</sup>, Sardar Khan<sup>3</sup>, Abid Ali<sup>1</sup>

<sup>1</sup>Department of Zoology Abdul Wali Khan University Mardan, KPK Pakistan; <sup>2</sup>Centre for Biotechnology and Microbiology University of Swat, Pakistan; <sup>3</sup>Department of Environmental Science, University of Peshawar



### Asar Khan

Department of Zoology Abdul Wali Khan University Mardan 2320...





DOI: dx.doi.org/10.17504/protocols.io.tb2eiqe

Protocol Citation: Asar Khan, Sumaira Shams, Saima Khan, Muhammad Iftikhar Khan, Sardar Khan, Abid Ali 2018. Modified ZN Staining Protocol . protocols.io <u>https://dx.doi.org/10.17504/protocols.io.tb2eiqe</u>

License: This is an open access protocol distributed under the terms of the <u>Creative Commons Attribution License</u>, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: September 07, 2018

Last Modified: September 07, 2018

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

- X Carbol-Fuchsin
- X Distilled Water
- X Methanol Sigma Aldrich Catalog #M3641
- 🔀 Disposable Latex Gloves, Medium, 100/Box Bio Basic Inc. Catalog #GL002M.SIZE.1PK
- X Methylene Blue Gold Biotechnology Catalog #M-680
- X Microscope slides
- 🔀 Compound Microscope
- 🔀 ethanol BBI Biotech
- 🔀 Acid Alcohol
- STEP MATERIALS
- X Carbol-Fuchsin
- 🔀 Acid Alcohol
- X Methylene Blue Gold Biotechnology Catalog #M-680

# Protocol materials Acid Alcohol In Materials, Materials, Step 6 Microscope slides Materials Methanol Merck MilliporeSigma (Sigma-Aldrich) Catalog #M3641 Materials Disposable Latex Gloves, Medium, 100/Box Bio Basic Inc. Catalog #GL002M.SIZE.1PK Materials Carbol-Fuchsin In Materials, Materials, Step 4 Distilled Water Materials Methylene Blue Gold Biotechnology Catalog #M-680 In Materials, Materials, Step 7 Compound Microscope Materials

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

- $\blacksquare$  3 mg (Amount of stool sample)
- 2 The slides were than placed on dryer with smeared surface upwards to air-dried them.

₿ 60 °C

(C) 00:10:00 minutes

3 The dried smear was fixed with absolute methanol.

(3-5 minutes) 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.

X 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.

X 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 airdry.

() 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