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Parasitology

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Modified Ziehl-Neelsen

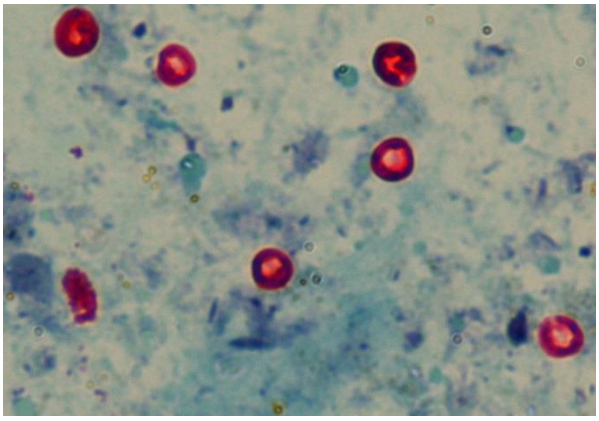
Use of the modified Ziehl-Neelsen stain for faecal smears has already been established for coccidian protozoa, in particular, oocysts of *Cryptosporidium* species, but it is also useful to confirm the presence of oocysts of *Isospora belli* and *Cyclospora cayetanensis*. (See Colour Plate 3, page 55)

Method

a) Faecal smears are made either directly from the stool sample or from the concentration deposit.
b) Allow to air dry.
c) Fix in methanol for 3 minutes.
d) Stain with strong carbol fuchsin for 15-20 minutes.
e) Rinse thoroughly in tap water.
f) Decolourise in acid alcohol (1% HCl in methanol) for 15-20 seconds.
g) Rinse thoroughly in tap water.
h) Counterstain with 0.4% malachite green (or methylene blue) for 30-60 seconds.
i) Rinse thoroughly and air dry.
j) Examine using x40 and x100 objectives.

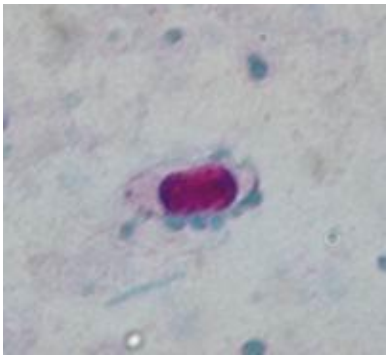
Oocysts of *Cryptosporidium parvum*

Oocysts of *Cryptosporidium parvum* do not concentrate well using standard concentration techniques and are identified using various staining techniques. Using the Modified Ziehl-Neelsen stain, the oocysts are acid-fast. However, staining within a smear and between specimens can vary from unstained, to partial red staining, to complete staining.



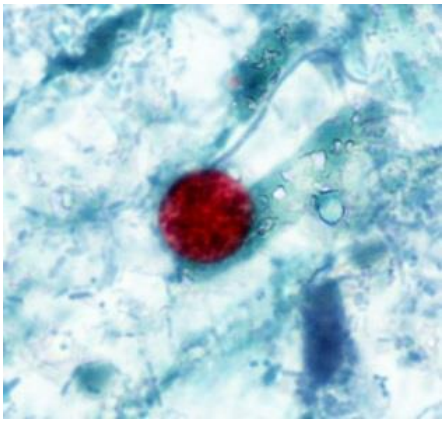
Oocysts of *Isospora belli*

The oocysts of *Isospora belli* can be demonstrated in faeces after formol-ether concentration. Alternatively, they can be seen in a faecal smear stained by modified Ziehl-Neelsen where the oocysts stain a granular red colour against a green background.



Oocysts of *Cyclospora cayetanensis*

The oocysts of *Cyclospora cayetanensis* can be seen in formol-ether concentrated stool samples. Alternatively they can be seen when stained with modified Ziehl-Neelsen where they exhibit variable staining; some cysts being acid fast whereas others appear as a round hole against the background Some are seen as glassy wrinkled spheres.



Phenol- auramine stain[3]

This stain can be used as an alternative to the modified Ziehl-Neelsen stain for staining oocysts of *Cryptosporidium parvum.*

Method

a) Make faecal smears as for ZN and fix in methanol.
b) Stain with phenol-auramine (Lemperts) for 10-15 minutes.
c) Rinse thoroughly in tap water.

d) Decolourise in acid alcohol. (as for ZN)

e) Rinse thoroughly in tap water.

f) Counterstain with 0.1% potassium permanganate for 30 sec.

g) Rinse thoroughly in tap water, allow to air dry.

Do not blot dry, many brands of blotting paper will fluoresce!

h) Observe the films with blue light under an incident light fluorescent microscope and low power followed by oil immersion x100 objective if oocysts are suspected.

The oocysts of *Cryptosporidium parvum* appear as bright yellow discs against a dark background. The oocysts of *Isospora belli* and *Cyclospora cayetanensis* do not fluoresce well using the phenol-auramine stain.

Summary of Diagnostic Characteristics

Microscopic Characteristscs	***Cryptosporidium parvum***	***Isospora belli***	***Cyclospora cayetanensis***
Size	4 - 6µ	20-33µ - 10 - 19µ	8 - 10µ
Identified in formol-ether concentrate by light microscopy	No	Yes	Yes
Identified by modified Ziehl-Neelsen	Yes	Yes	Yes
Identified by Phenol Auramine stain	Yes	Variable	No