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Section: Parasitology Manual	Subject Title: Laboratory Procedures for Stool Examination	
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Sequential Haematoxylin/Acid Fast Stain For Routine O & P Including *Cryptosporidium* & *Cyclospora*

PRINCIPLE

Haematoxylin stain is a useful technique, however several organisms of interest such as cryptosporidium and cyclospora do not stain well with this technique. To overcome this limitation an acid fast stain can be ordered on suspected crypto or cyclospora samples, or alternatively a sequential stain can be used. The addition of an acid fast stain causes these organism to stain a bright red, however the depth of stain of *Cyclospora* can be variable. The sequential stain is especially appropriate in situations were it is suspected that crypto and cyclospora levels are elevated in the general population.

SPECIMEN

Prepare fecal smears as in the **permanent stained smear methods**.

SAFETY

Be careful when handling acid solutions and ammonia. Picric acid becomes explosive if the solution is allowed to dry out.

MATERIALS

- See **Permanent Stained Smear** method.
- Kinyoun stain (commercial product, PML)
- Decolorizer (commercial product, PML)
- Hematoxylin stain (commercial product, VWR)
- Picric acid (commercial product, VWR)

PROCEDURE

Place slides into the following solutions for the times indicated; do not stain on a rack:

- | | | |
|----|--------------------------|-------------|
| 1. | 70% alcohol | 6 minutes |
| 2. | Tap water | 1 minutes |
| 3. | Kinyoun stain | 5 minutes |
| 4. | Tap water rinse | 3 minutes |
| 5. | Acid-alcohol decolorizer | 2-4 minutes |
| 6. | Tap water rinse | 2 minutes |

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7.	Haematoxylin stain	8 minutes
8.	Distilled water rinse	20 seconds
9.	Picric acid	3-5 minutes
10.	Running water wash	12 minutes
11.	70% alcohol + ammonia	5 minutes
12.	95% alcohol	3 minutes
13.	100% alcohol	3 minutes
14.	xylol	3+ minutes
15.	xylol	1+ minute

QUALITY CONTROL

- Ensure that the microscope has been calibrated in the last year or any time the optics have been altered and that the results of the calibration are displayed on the microscope base.
- A positive control slide containing *Cyclospora* should be stained at the same time as the unknowns. The unknown slides cannot be read if the control slide is unsatisfactory.

REPORT

- All parasites including *Cyclospora* and *Cryptosporidium*.

PROCEDURE NOTES

1. *H. diminuta* will stain bright red with a clear background. The size of this organism will be reduced because of the stain/fixation process.

LIMITATIONS OF PROCEDURE

2. Light infections with cryptosporidia and cyclospora may be missed. Immunoassays may be more sensitive.
3. Multiple specimens must be examined since the number of oocysts may vary day to day.
4. Other organisms that stain modified acid fast positive including *Nocardia*, and *Microsporidia* may be difficult to identify.

AUTHOR

Ian Crandall

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REFERENCES

Garcia LS, Bruckner DA. Diagnostic Medical Parasitology. 3rd Edition. ASM Press, Washington DC. 1997.

Palmer, J. Sequential Kinyoun/hematoxylin staining procedures for the Cryptosporidium and other intestinal parasites. Microbiol Letter March 1st, 1991