

Evaluation Of Microscopic Staining Techniques For The Diagnosis Of Opportunistic Protozoan Infections In A Developing Country

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Abstract

Trichome staining method was superior to other techniques in detecting opportunistic protozoan infections: 35.7% for *Cryptosporidium* sp, and 50% for *Microsporidium* sp. The Acid Fast (Carbol Fuchsin) stain was useful only in detecting the oocysts of *Cryptosporidium* sp.. With 35.7% positivity. Laboratories in developing countries can put Tricrome stain in their priority purchase.

INTRODUCTION

The prevalence of intestinal parasites has been studied extensively in both rural and urban areas of Nigeria^{1,2,3}. Routine diagnosis of intestinal parasitic infections is usually performed by a simple smear in normal saline or iodine stain solution using light microscopy⁴. More recently, some unusual protozoans such as *Cryptosporidium* Spp, *Isospora belli*, *Microsporidium* Spp, *Cyclospora cayentensis* and *Blastocystis hominis* have become important aetiological agents of diarrhea in children and patients with some type of immune compromised conditions e.g. HIV/AIDS⁵. In these immunocompromised patients, auto-limiting diarrhea in immunocompetent persons may cause profuse diarrhea, malabsorption syndrome, anorexia, weight loss; low grade fever and abdominal pains or cramps^{6,7}. With these new frequent presentations, a new pattern of diagnosis of intestinal infections is therefore required. However, in most laboratories, the diagnosis of these unusual protozoan is not part of the routine wet smear ova and parasite light microscopy, but involves the use of special stains.⁸ The non-use of these stains usually present a challenge in the diagnosis of these uncommon parasites in most developing countries. Therefore, it became necessary to evaluate common microscopic staining techniques available in our laboratory, with the view of recommending a stain or combination of stains that has a broad range application for the various parasites.

THE STUDY

70 diarrhoeal stool samples (each pooled from three consecutive days) obtained from various patients, including HIV/AIDS patients who were attending various clinics in Lagos, Nigeria were processed for routine microscopic examinations of ova and parasites. Saline and Iodine wet mount, and permanent slides of Giemsa, Carbol Fuchsin (Kinyoun-modified acid fast) and Weber's modified Trichrome stains were also made. Faecal smears on slides and formal-ether concentration methods were prepared for each stool samples using standard procedures^{9,10}. The micrometer was used for the accurate measurement of oocysts, cysts, and ova that were seen. The 100X oil immersion lens was employed in all cases to demonstrate the morphology of oocysts. Informed consent was obtained from selected patients after explaining the aim of the study. These patients were already enrolled in an on-going HIV/AIDS clinics and treatment programme.

Intestinal opportunistic protozoans, particularly *Coccidian* and *Microsporidium* spp. are emerging infections that are important complications of intestinal morbidity in immune compromised patients¹⁰. The laboratory diagnosis of these parasites in our region involves mainly light microscopy, and therefore places a high responsibility on the laboratory scientist. The use of light microscopic stains for diagnosis of these infections has improved greatly over the years, following the modification of some basic staining

techniques. This is of importance to laboratories in developing communities, where lack of electron microscopy and molecular biology facilities for the diagnosis of opportunistic infections are lacking. However, the problems of the unavailability of the stains and the cost of purchasing or ordering large varieties are issues facing laboratories in most developing countries.

Figure 1

Table: Assessment of four Laboratory Stains for 'Broad Range' Use in the Microscopic Diagnosis of Coccidian and Microsporidial Parasites.

Total Number of Stools examined = 70

S/No	Stains	<i>Cryptosporidium</i> / <i>Isospora</i> No. (%)	<i>Microsporidium</i> sp. No. (%)
1.	Iodine	-	-
2.	Giemsa	2(3)	-
3.	Carbol Fuchsin	25(35.7)	-
4.	Weber's Trichrome	25(35.7)	35(50)

Note: The negative stool samples were also part of the 70 samples examined.

In this study, Weber's modified Trichrome stain using chromotrope 2R and phosphotungstic acid clearly differentiated the spores of microsporidium in 50% and 35.7% of oocysts of *Cryptosporidium* of the positive samples (Table). Carbol fuchsin stains, on the other hand, diagnosed 35.7% of the oocysts of *Cryptosporidium* species but less efficient in diagnosing *Microsporidium* sp. Although, Giemsa stain has been reported useful for routine diagnoses of cryptosporidium, our result showed that Giemsa stain, with 3% positive is impractical for the diagnosis of *Cryptosporidium* sp. Saline concentration and iodine staining techniques for wet smear microscopy, while lending itself useful in routine microscopy for ova and cysts of some parasites are grossly in-effective for the diagnosis of Coccidian and *Microsporidium* sp. In general, the chromotrope 2R and Carbol fuchsin diagnosed *Cryptosporidium* *pervum* in the same stool specimen, which showed equal sensitivity and specificity.

CONCLUSION

Weber's Trichrome staining techniques could be prioritized in the purchase of stains for the diagnosis of *Cryptosporidium* species, *Isospora belli*, and *Microsporidium* sp. The availability and use of this stain will provide useful information for better management of diarrhea especially

among the immune compromised patients especially in cases of HIV/AIDS conditions.

SUMMARY

Trichrome stain is adequate to diagnose coccidian opportunistic parasites in developing countries where resource is constrained and should be considered by laboratories operating a tight budget

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