A comparison of five staining methods for detection of Cryptosporidium oocysts in faecal specimens from the field

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Five staining methods for identification of Cryptosporidium oocysts in faecal smears were evaluated using samples obtained from asymptomatic south Indian villagers of all ages. The safranine-methylene blue technique was used as the gold standard and compared with 2 methods each using auramine and mepacrine, with potassium permanganate and carbol fuchsin as counterstains. All five methods identified all samples containing cryptosporidium oocysts, however false positive identifications were obtained by the standard auramine-phenol-potassium permanganate method. The auramine-carbol fuchsin and the mepacrine-carbol fuchsin techniques are the simplest and most rapid of the five methods compared. The mepacrine-carbol fuchsin technique is recommended for screening large numbers of stools, since it does not stain yeasts, is cheaper and less toxic than auramine.

Key words Cryptosporidium oocysts-staining methods

Cryptosporidiosis is a zoonotic disease caused by a parasitic protozoan belonging to the coccidial genus, Cryptosporidium. A number of methods have been found to be satisfactory when watery diarrhoea is present and the specimen contains numerous oocysts. However, when the faecal specimen is formed or semi-formed, and oocysts are scarce, then selection of the method used for detection becomes most important. A Joint Working Group has recently recommended screening samples by an auramine technique and confirmation of putative positives by staining a fresh faecal smear by the modified Ziehl-Neelsen method.

We have earlier used the safranine-methylene blue technique, a modified Ziehl-Neelsen stain to identify Cryptosporidium oocysts in the hospital setting. In this study on samples from the field we compared five staining methods for sensitivity, specificity and ease of performance. These are the safranine-methylene blue (SMB) stain, the auramine-phenol fluorescent stain, the auramine-carbol fuchsin fluorescent stain, a mepacrine-potassium permanganate stain and a mepacrine-carbol fuchsin stain, of which the latter two are modifications of a recently described new staining technique which uses mepacrine as the fluorescent dye, the advantage being that yeasts are not stained.

Material & Methods

Subjects: Five hundred stool samples were collected between May 1994 and June 1995 from people of all ages from a stratified random sample of a village 25 km from Vellore. None of the individuals had diarrhoea or antibiotic treatment in the one month prior to the collection of the sample.
Stool examination: Samples were transported on ice and processed within two hours of collection. The modified formal-ether technique was used to concentrate approximately 2 g of stool and three smears were made on glass slides from each specimen. All stains were obtained from Difco Laboratories (Detroit, USA) or Sigma (St. Louis, USA).

For the first 300 samples, two slides were fixed in acid-methanol and stained by the safranine-methylene blue technique and the auramine-phenol technique. The last slide was heat-fixed and stained with auramine-phenol and counterstained with carbol fuchsin without decolourisation.

For the next 200 samples, one slide was heat-fixed and stained by the auramine-carbol fuchsin technique as described above. The other two slides were fixed in 10 per cent formalin for 2-3 min and stained with aqueous mepracrine. One slide was decolourised with alcohol and counterstained with potassium permanganate, and the other slide was counterstained with carbol fuchsin without decolourisation. Fresh smears were prepared from all samples found to be positive by the fluorescence techniques and re-examined by the SMB method.

All SMB stained slides were examined under a 100X oil immersion lens. The slides stained with auramine and mepracrine were examined under 20X and 40X lenses of an epifluorescence microscope (Olympus, Japan).

Five smears were also prepared from cultures of Candida albicans and Saccharomyces cerevisiae and examined by each of the five techniques described above.

Results

All the samples received were formed or semi-formed in consistency. In the first 300 samples examined, staining by the SMB technique revealed 34 specimens (11.3%) that had Cryptosporidium oocysts. Each slide took about 5 min to examine.

The standard auramine-phenol stain resulted in 38 samples being identified as positive. However, on re-examination of SMB stained smears from these subjects, four were found to be yeast cells that had been misidentified. Although sensitivity was 100 per cent, the specificity of this technique was 98.4 per cent using the SMB method as the gold standard (Table). Despite the decolourisation step, background fluorescence was present in the slides stained by this technique, but examination time for each slide was only about 2 min.

The auramine-carbol fuchsin method also identified the 34 positive samples, all of which were confirmed as Cryptosporidium on re-examination by the SMB technique, giving a sensitivity and specificity of 100 per cent. Examination of these slides was easier than in the standard technique because of the lack of background fluorescence, although the time spent in examining the slides was the same in both fluorescence methods. Without the decolourising step this technique was simpler and shorter.

In the next 200 samples, the auramine-carbol fuchsin technique identified Cryptosporidium oocysts in 18, all of these were confirmed by the SMB method. All 18 were also identified using the mepracrine staining methods, with no false positive identification, giving a sensitivity and specificity of 100 per cent for both methods. However, background fluorescence in the slides counterstained with potassium permanganate was high and yeasts seemed to stain faintly with mepracrine, although there was a clear differentiation between Cryptosporidium oocysts and yeasts, both in intensity of staining and morphology. With carbol-fuchsin, the background fluorescence was less, slides were easier to examine and no staining of yeasts was seen. The time taken to examine each mepracrine stained slide was about 2 min.

Discussion

There is a high prevalence of Cryptosporidium oocysts in south Indian children with diarrhoea and
in matched controls. We have evaluated five methods of examining faecal smears in order to carry out further field studies, using samples from asymptomatic controls from this population. All five techniques were equally sensitive, but the auramine-phenol stain yielded a few false positives. The fluorescence techniques were quicker and more suited to field conditions to detect oocysts. The method using mepacrine has not previously been used to evaluate human faecal samples. Mepacrine does not stain yeasts, is cheaper and less toxic than auramine, and can be used with carbol fuchsin as a counterstain without a decolourisation step, thereby making it suitable for field surveys where large numbers have to be screened.

A joint working group has recommended that a preliminary screen of faecal samples be done by the auramine-carbol fuchsin stain followed by confirmation by a modified Ziehl-Neelsen stain. Based on our experience, we would recommend that mepacrine-carbol fuchsin be used as the routine first stain for the rapid identification of oocysts rather than auramine-carbol fuchsin, followed by a confirmatory stain on putative positive samples.

References


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