Comparison of Weber Green and Ryan Blue Modified Trichrome Staining for the Diagnosis of Microsporidial spores from Stool Samples of HIV-Positive Patients with Diarrhoea

Dear Editor,

Microsporidia is increasingly being reported in the recent years in HIV-positive patients with diarrhoea.[1,2] Electron microscopy, though a gold standard for detection of microsporidial spores,[3] can only be used in sophisticated laboratories. Intestinal biopsy, though better, is not advocated in HIV-infected patients with diarrhoea because of its invasive nature.[3] Therefore, trichrome staining still remains the most commonly used method for detection of microsporidial spores in routine laboratories. Therefore, on 30 stool samples of HIV-positive patients with diarrhoea, modified trichrome staining, i.e., Chromotrope 2R staining, was performed in duplicate — one method using fast green (Weber Green method)[4] and the other method using aniline blue (Ryan Blue method).[5] The procedure of staining was similar for both. Smears were examined under oil immersion lens for pink-coloured spores 1 to 2 µm in diameter. Background appeared green in case of fast green and blue when aniline blue was used. Photographs of microsporidial spores by two staining methods are shown (Fig.).

Weber Green method could detect microsporidia in 10% of the samples, whereas Ryan Blue method detected the same in 6.67% of the samples. By validity method, sensitivity and specificity of Ryan Blue method were 60% and 50.9% respectively taking Weber Green method as the gold standard. The positive predictive value was 10%, and negative predictive value was 93.3%.

Ryan et al.[5] reported a good contrast between microsporidial spores by using aniline blue as an alternative to fast green in Chromotrope 2R stain. They observed that fast green is quite pale and fades rapidly as compared to aniline blue, and use of aniline blue has the advantage of yeast cells and pseudohyphae staining greyish blue. In contrast, results of this study showed better results with fast green staining. However, the sample size was very small and further study is required in this regard. Other modifications of trichrome stain, like Gram Chromotrope staining, need to be tried.

References

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