

Images in Clinical Tropical Medicine

Ziehl–Neelsen in Schistosomiasis: Much More than Staining the Shell and Species Identification

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The Ziehl–Neelsen (ZN) stain, also known as the acid-fast stain, has been reported to be helpful in detection and identification of *Schistosoma* eggs.^{1,2} In histological sections, *Schistosoma mansoni* egg shells appear as ZN positive and *Schistosoma haematobium* shells as ZN negative.^{1,2} The staining target of the responsible ZN component (carbol-fuchsin) in the shell is unknown. Because carbol-fuchsin is supposed to stain mycolic acids in the mycobacterial cell wall,³ unidentified substances in the egg shell were proposed as target.² However, in histopathological examination, biopsies are exposed to concentrated ethanol and xylene during their embedding. This may alter the egg shell and allow some unusual staining reactions not seen in eggs from other sources. In fact, the ZN stain is not consistently positive in feces.³ In addition, in some intact *S. mansoni* eggs, the shell was found to be only weakly ZN positive while the miracidium was found intensely positive.³ Fuchsin is a known nucleic acid stain,⁴ and it was already shown that mycobacteria with insufficiently retained carbol-fuchsin may be invisible in bright-field microscopy. Yet, they can be easily detected because of a strong red fluorescence when excited with green light.⁵

A smear of *S. mansoni* eggs was prestained with the nucleic acid stain 4',6-diamidine-2'-phenylindole dihydrochloride

(DAPI) and then stained with the common ZN procedure. The smear was observed (40× objective) using bright-field and fluorescent microscopy where carbol-fuchsin fluoresces red (ZN-fluo)⁵ and DAPI fluoresces blue (DAPI-fluo) (Figure 1). In bright-field microscopy, the shell appears to stain very little, whereas the miracidium within the intact egg and outside the egg appears acid-fast negative, apparently only retaining the counter-stain methylene blue. Contrary to this, fluorescent microscopy shows strong staining of the miracidium with carbol-fuchsin (ZN-fluo) and DAPI (DAPI-fluo). The co-localization (merged) reinforces the idea that carbol-fuchsin is indeed a nucleic acid stain.⁴ Because acid-fast stains and low-cost light-emitting diode fluorescent microscopy are now commonly used in many regions where schistosomiasis is endemic, it may be the time to revisit the staining mechanisms of acid-fast stains⁴ and investigate the use of these stains for their capacity to improve the detection of *Schistosoma* eggs.

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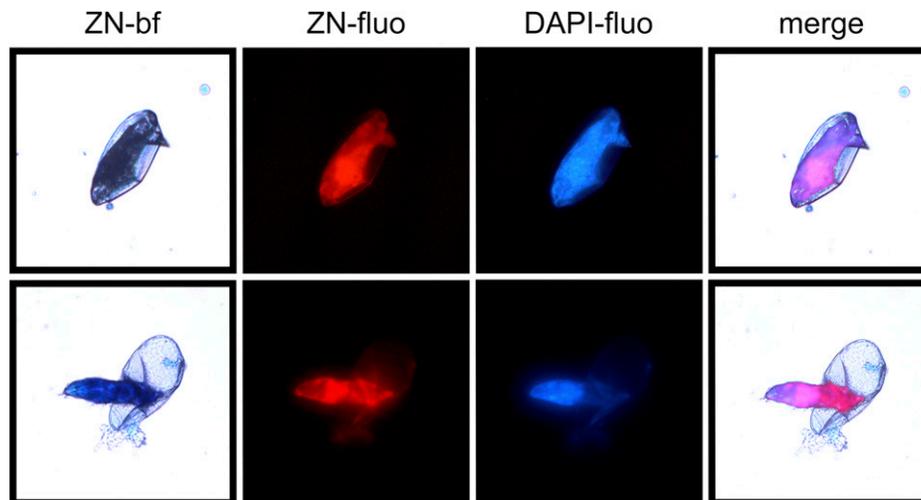


FIGURE 1. Bright-field and fluorescent image of ZN-stained *S. mansoni* eggs.

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