Using Fluorescence as a Death Assay for Angiostrongylus cantonensis Nematode

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Rat lungworm disease (angiostrongyliasis), caused by human infection of Angiostrongylus cantonensis, is a dilemma that plagues inhabitants of tropical climates globally. The lifecycle of rat lungworm usually is limited to rats and slugs/snails, however humans may be incidental hosts if they ingest the larvae or slime via raw or undercooked vegetables. Once humans are infected, the larvae invade the brain and often cause serious neurologic symptoms. As rat lungworm infections become more prevalent, there is growing incentive for developing an effective method to both treat angiostrongyliasis and develop a vegetable wash to help prevent human infections. This experiment analyzed the effectiveness of fluorescent staining as a death assay for A. cantonensis. The cell-permeant fluorescent molecule Propidium Iodide was investigated as a stain that would only penetrate the surface membrane of dead larvae, and the fluorescent molecule Hoechst was investigated as a stain that would penetrate the surface of live and dead larvae. Images of fluorescence were collected from the Operetta, a high-content imaging system, and were then processed in ImageJ, an image analysis software. Total fluorescence area and fluorescence intensity data was collected by setting threshold ranges for pixels. As hypothesized, Propidium Iodide only stained dead larvae, and Hoechst stained live and dead larvae. Therefore, fluorescence staining death assays using Propidium Iodide and Hoechst is effective, and can be used to determine the success of killing larvae with household washes.