

An Advanced Approach to Detect and Enumerate Soil-Transmitted Helminth Ova from Wastewater

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Abstract : Parasitic diseases have a devastating, long-term impact on human health and welfare. More than two billion people are infected with soil-transmitted helminths (STHs), including the roundworms (*Ascaris*), hookworms (*Necator* and *Ancylostoma*) and whipworm (*Trichuris*) with majority occurring in the tropical and subtropical regions of the world. Despite its low prevalence in developed countries, the removal of STHs from wastewater remains crucial to allow the safe use of sludge or recycled water in agriculture. Conventional methods such as incubation and optical microscopy are cumbersome; consequently, the results drastically vary from person-to-person observing the ova (eggs) under microscope. Although PCR-based methods are an alternative to conventional techniques, it lacks the ability to distinguish between viable and non-viable helminth ova. As a result, wastewater treatment industries are in major need for radically new and innovative tools to detect and quantify STHs eggs with precision, accuracy and being cost-effective. In our study, we focus on the following novel and innovative techniques: -Recombinase polymerase amplification and Surface enhanced Raman spectroscopy (RPA-SERS) based detection of helminth ova. -Use of metal nanoparticles and their relative nanozyme activity. -Colorimetric detection, differentiation and enumeration of genera of helminth ova using hydrolytic enzymes (chitinase and lipase). -Propidium monoazide (PMA)-qPCR to detect viable helminth ova. -Modified assay to recover and enumerate helminth eggs from fresh raw sewage. -Transcriptome analysis of *ascaris* ova in fresh raw sewage. The aforementioned techniques have the potential to replace current conventional and molecular methods thereby producing a standard protocol for the determination and enumeration of helminth ova in sewage sludge.

Keywords : colorimetry, helminth, PMA-QPCR, nanoparticles, RPA, viable

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