

Overcoming Molecular Sample Processing Limitations

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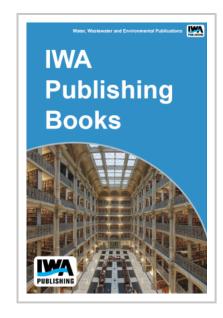
This report on Overcoming Molecular Sample Processing Limitations is split into three sections:

(a) New Platform Technologies

Rapid, low-cost extraction of DNA from bacterial cultures and colonies was investigated using four reagents: Instagene matrix (Bio-Rad, Hercules, CA); Isocode paper (Schleicher and Schuell, Keene, NH); PrepMan (Applied Biosystems, Foster City, CA); and Xtra Amp tubes (Ansys Diagnostics, Lake Forest, CA).

(b) RNA and DNA Extraction Strategies

The purpose of this study was 1) to develop standardized protocols for the rapid (less than 8 hours) quantitative PCR detection of total intact Cryptosporidium and potentially



infectious enteroviruses in wastewater samples, 2) to determine the detection sensitivities of the methods using seeded wastewater samples, 3) to provide statistical evaluation for the determination of reproducibility, accuracy of quantitation, and variability of the methods, and 4) to investigate the feasibility of the developed methods using unseeded wastewater samples. Concentration methods for two different volumes of Cryptosporidium and enterovirus samples were evaluated. This allowed a comparison of the methods based on their ease of use, cost and performance.

(c) Fiber-Optic Biosensors

This study investigated the use of a commercially available fiber optic biosensor (Analyte 2000TM, Research International, Woodinville, WA) for near real-time detection of the pathogens E. coli O157:H7 and Salmonella typhimurium in wastewater.

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