

Rapid and low-cost DNA extraction procedure for PCR-based detection of pathogenic organisms in environmental waters

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Waterborne diseases are prevalent in developing countries including Sri Lanka causing four-fifth of all illnesses. Current culture-based survey and detection methods are expensive, labor-intensive, time consuming and frequently result in erroneous or inconclusive results. Two rapid and low-cost molecular methods based on chaotrophic properties of guanidinium isothiocyanate (GITC) were modified and tested. In the first procedure, GITC was used in the lysis buffer and in the second procedure it was used in the protein precipitation step. DNA yields and purity were estimated by UV spectrophotometry and suitability of purified DNA for downstream applications was tested by PCR amplification with uidA primers specific for *E. coli* gene β -D glucuronidase and Bac32F and Bac708R primers specific for 16SrRNA gene of *Bacteroides* species. DNA yields estimated by the pellet size and agarose gel electrophoresis was higher in method 1 but UV spectrophotometric readings overestimated yields probably due to continued presence of contaminants including guanidinium and proteins although not detected in UV spectrum study. Presence of these contaminants however had no inhibitory effect on PCR amplification with both methods giving specific PCR products with both primer pairs. Results indicate method 1, previously described for detection of pathogenic microorganism in clinical samples is superior to method 2 producing higher yields and purify of DNA and higher PCR yields. Some modifications to both methods are needed when dealing with water containing high levels of algae.