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Development of novel seminested polymerase chain reaction assays for detecting toxigenic *Vibrio cholerae* and *Shigella* spp. in water

by

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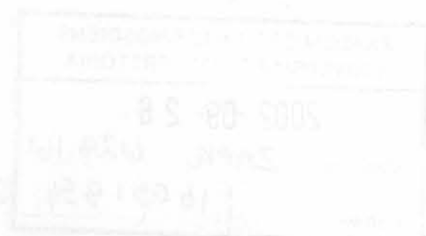

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DECLARATION

I declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

Martella du Preez

Date

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SUMMARY

Develpoment of novel seminested polymerase chain reaction assays for detecting toxigenic *Vibrio cholerae* and *Shigella* spp. in water

by

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Rapid and sensitive polymerase chain reaction (PCR)-based assays were developed for the detection of toxigenic *Vibrio cholerae* and virulent *Shigella* spp. in environmental water samples. In contrast to conventional nested and seminested PCR assays, the newly developed pit-stop seminested PCR assays are performed in a total of 30 amplification cycles. Oligonucleotide primers were designed to specifically amplify sequences within the cholera toxin gene (*ctxA*) of *V. cholerae* and the invasion plasmid antigen gene (*ipaH*) of virulent *Shigella* spp. By using agarose gel electrophoresis for detection of the PCR-amplified products, a detection limit of 1.3 x 10^3 cfu of *V.cholerae* and 1.6 x 10^3 cfu *of Shigella flexneri* were obtained from crude cell lysates. However, by coupling the PCR assays with an enrichment culture procedure, the sensitivity of the

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assays was improved and as few as 4 cfu and 1.6 cfu of *V. cholerae* and *S. flexneri* organisms, respectively, could be detected in pure culture. Analysis of seeded environmental and drinking water samples yielded detection limits of as few as one *V. cholerae* organism/ml and less than 14 cfu/ml *S. flexneri* in some samples.

The basic PCR detection protocol was modified to incorporate a membrane filtration step in order to concentrate large volumes of water samples. This allowed for the detection off low numbers of viable *V. cholerae* and *Shigella* spp. in naturally contaminated waters. By making use of seeded environmental, and drinking water samples, detection limits of 1 cfu/100 ml of *V. cholerae* and 8 cfu/100 ml of *Shigella* cells were obtained. The detection protocol was successfully applied to the analysis of naturally contaminated environmental water samples. The results obtained in this study indicate that the newly developed pit-stop seminested PCR assays, combined with an enrichment step, fulfills the requirements of simplicity and sensitivity for use in an environmental laboratory and may represent significant tools in monitoring water sources for the presence of *V. cholerae* and *Shigella* spp.

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