Development and implementation of a rapid real-time polymerase chain reaction assay for the detection of toxigenic *Vibrio cholerae* in water

Neil Leat* and Monique Grundlingh
Microbiology Laboratory, Analytical Services, Rand Water, PO Box 3526, Vereeniging, 1930, South Africa

ABSTRACT

Assays which use real-time polymerase chain reaction (PCR) technology can be developed for the rapid identification of genetic sequences carried by waterborne pathogens. Rand Water has established facilities within which a selection of PCR assays will be developed. This paper reports on the optimisation and validation of the first assay to be implemented. This assay facilitates the detection of the *ctxA* gene of toxigenic *Vibrio cholerae* (*V. cholerae*) strains. The assay also includes an internal process control in the form of an *Escherichia coli* (*E. coli*) strain carrying a single genomic copy of the *gfp* gene from *Aequorea victoria*. Establishment of the assay required the selection of suitable PCR primers and probes for both the *ctxA* and *gfp* genes. This was followed by an optimisation phase where ideal PCR cycling conditions and primer/probe concentrations were established. A validation phase established the performance parameters of the assay. Parameters assessed included: limit of detection, sensitivity, specificity, reproducibility and robustness. The validation was conducted using potable water, surface water and sewage effluent matrices. The process has resulted in the establishment of a robust assay for the detection of toxigenic *V. cholerae* strains within 24 hours after samples are received.

Keywords: real-time PCR, *Vibrio cholerae* O1, *ctxA* gene, validation

INTRODUCTION

Close to 200 serotypes of *V. cholerae* have been identified; however, significant cholera epidemics have been associated almost exclusively with the toxigenic O1 and O139 serotypes (Kaper et al., 1995). Ingestion of toxigenic *V. cholerae* results in severe watery diarrhoea and may be fatal in the absence of suitable medical intervention. While it would be unlikely for *V. cholerae* to bypass well-managed potable water treatment systems, rigorous monitoring provides additional confidence that safe water is being produced.

To date Rand Water has used a conventional culture-based approach for the identification of *V. cholerae* in water samples. This approach involves enrichment of *Vibrio* species in samples using alkaline peptone water (APW), with incubation at 36°C for 16 to 18 h. This is followed by isolation of presumptive *Vibrio cholerae* strains on thiosulfate citrate bile salts sucrose agar (TCBS). Presumptive isolates are confirmed using biochemical tests followed by a serological assessment to identify the *V. cholerae* O1 antigen. While this process has been effectively implemented, it takes a number of days to complete.

Real-time polymerase chain reaction (PCR) technology provides a more efficient alternative to the classical culture-based approach. The key advantage is that virulence genes can be directly and rapidly detected in APW enrichments. To date, a plethora of real-time PCR assays for the detection of *V. cholerae* have been published. Three examples are the assays published by Huang et al. (2009), Koskela et al. (2009) and Mehrabadi et al. (2012). The assay published by Huang et al. (2009) is a quadruplex real-time PCR assay targeting 4 genes (*ctxA*, *hlyA*, O1-*rfb* and O139- *rfb*). The assay published by Koskela et al. (2009) targets 2 virulence genes (i.e. *toxR* activator and *ctxA*). The assay published by Mehrabadi et al. (2012) targets the *ctxA*, *tcpA* and *ompW* genes.

While the elaborate multiplex assays typically published in the literature are technologically impressive, almost none of these include a single process control which is carried through both nucleic acid extraction and PCR amplification steps. In addition, most target several virulence genes which may introduce unnecessary complexity when the principal concern is to detect toxigenic *V. cholerae* in the simplest way possible.

The requirement within Rand Water was to establish a simple robust assay that would target one toxigenic *V. cholerae* virulence gene as well as one process control gene, carried through both nucleic acid extraction and PCR amplification steps. By selecting the simplest assay possible the subsequent validation and implementation of the assay would be more readily achieved. For the purposes of this study the *ctxA* gene was selected as the primary target. The *ctxA* gene encodes the cholera toxin and is one of the most important factors responsible for severe diarrhoea Kaper et al. (1995).

For the present study a process control was developed based on the system described by Murphy et al. (2007). The process control utilises an *Escherichia coli* BW 31004 strain which has been engineered to carry a single copy of the *gfp* gene on its genome (Zhou et al., 2004). The *gfp* gene encodes the green fluorescent protein of the jellyfish *Aequorea victoria*. The *gfp* gene was considered unlikely to be present in environmental samples analysed by Rand Water.

The implementation of the current assay required the selection of suitable PCR primers and probes for both the primary target gene (*ctxA)* and the process control gene (*gfp)*. This was followed by an optimisation phase where ideal PCR

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* To whom all correspondence should be addressed.
☎ +27 16 430-8403; fax: +27 16 455-2055;
e-mail: nleat@randwater.co.za

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