Same-day Beach Closure Decisions Using Real-time Quantitative PCR Assay: Detection of E. coli in Milwaukee Area Beaches

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Abstract

Objective: To compare the real-time quantitative PCR (qPCR) assay with conventional methods for same-day detection of microbial pollution indicators during beach monitoring in Milwaukee.

Study Design: Beach water samples are routinely examined at the City of Milwaukee Health Department Laboratory (MHDL) for the presence and quantification of E. coli. The qPCR assay was compared to conventional methods for same-day decisions.

Methods: A significant correlation was found between the qPCR and conventional methods (C.E. value > 200 but MPN < 235) for E. coli monitoring in Milwaukee.

Results:

- A significant correlation was found between the qPCR and conventional methods.
- Same-day beach closure decisions with qPCR results were possible and followed in 24 hours by Colilert.

Conclusions:

- The qPCR assay was found to be comparable to conventional methods for same-day beach closure decisions.

Introduction

Swimming associated illnesses rarely occur as a result of exposure to enteric bacteria, viruses and protozoa. Several indicator bacteria such as E. coli are ubiquitous; therefore, it is difficult to differentiate between viable and non-viable cells. However, the presence and quantitation of indicator enteric bacteria found in beach water samples may indicate the presence of enteric pathogens (1).

Materials and Methods

Swimming-associated illnesses can be caused by a variety of enteric pathogens, which can be detected using both culture-based and molecular methods. Culture-based methods are typically used for detecting E. coli, which is the organism specifically targeted in this study. Molecular methods are used to detect specific nucleic acid sequences and are typically more sensitive than culture-based methods.

Results

- A significant correlation was found between the qPCR and conventional methods for E. coli monitoring in Milwaukee.

qPCR Assay Set up

- DNA Extraction: For standard curve, Crude DNA extracted by bead beating was purified using commercially available Zymo DNA Clean and Concentrator kit. For lorem, calibration curve samples, DNA extractions were performed using commercially available Zymo Research QIAamp DNA kit.

References:


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Limitations

- The qPCR assay may not be as sensitive as culture-based methods in all environments.
- The qPCR assay may not be as sensitive in high-competitive environments.
- The qPCR assay may not be as sensitive in low-competitive environments.

Conclusions

- Same-day beach closure decisions can be made using qPCR data.
- Culture-based methods can be used as a backup if qPCR data are not available.
- The qPCR assay can be used to improve beach monitoring and management.

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