Introduction
In Response to Congressional legislation, the US EPA is currently evaluating methods for rapidly determining the densities of fecal indicator bacteria (FIB) in recreational waters. A review of epidemiological studies conducted by the US EPA found that density estimates of total Enterococci, as determined by quantitative polymerase chain reaction (qPCR), were highly associated with an increased incidence of swimmer related illness. The analysis of Enterococci by qPCR is, therefore, being considered for approval as part of the development of new/revised health-related recreational water quality criteria. Potential widespread implementation of qPCR may be hindered by uncertainty in the ability to reproduce test results within and between laboratories.

Objectives
The primary objectives of the study were to: 1) measure the variability in standard curve constructs between different technicians within the same lab, 2) assess variability in standard curves between different labs using the same calibrator source material, 3) examine the reproducibility of standard curve results from year to year using archived cell preparations, and 4) determine the deviation of calibrator results based on calibrator cells source material.

Methods
Analyzed calibrator source materials included: lab prepared fresh cells, lab prepared archived frozen cells (2007), referee prepared cell impregnated filters (UNC-Chapel Hill), and commercially prepared cells (Bioballs™, BTF 12897-550).

All of calibrator source materials were analyzed by qPCR using US EPA Method A: Enterococci in Water by TaqMan® Quantitative Polymerase Chain Reaction (qPCR) Assay (formerly Method 1808).

Results and Discussion

Calibration curve reproducibility within the same lab:
ANOVA conducted on the R² value derived from standard curves prepared by three analysts within the same lab using fresh cells demonstrated no significant difference (p = 0.510).

Calibration curve reproducibility between different labs:
Standard curves produced by different labs using the same cellular material demonstrated a high degree of similarity σ = 2.47E-3 of R².

Results and Discussion (cont.).

Calibrator cells stability:
• Frozen cells prepared in 2007, stored at -80 °C, and re-analyzed in 2010 deviated little from their original value [standard curve R² value of 0.9933 (2007) vs. 0.9980(2010)].

• The difference between using fresh cells (any) over frozen archived cells was insignificant (p = 0.794).

Conclusions
Study results demonstrate that while commercially prepared products reduce variability, an acceptable degree of precision can be obtained within and between labs using a variety of materials via the evaluation of standard curve preparations.

Lab prepared frozen cell stocks, when stored at -80 °C, retained their stability over a 3-year period. Therefore, use of frozen cells would reduce standard curve preparation time and the associated supply costs.

qPCR methods enumerate FIB, such as Enterococci, from source samples via interpolation from standard curves. A lack of precision in the development of standard curves will lead to erroneous values, resulting in either taking unnecessary regulatory action (closing a recreational water body to swimming in the absence of health risk) or lapses in public health protection due to false negative results. Therefore, laboratory studies evaluating the precision and accuracy of calibrator source material must be required when implementing molecular-based analytical methods.

References


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