Quantitative-PCR for Rapid Indicator Measurement at Inland Waters

J. L. Kinzelman1, J. S. Lavender2, R. T. Noble3, A. D. Blackwood4, S. Dorevitch5, R. Bushon5
1City of Racine Health Dept., Racine, WI, 2USEPA, Cincinnati, OH, 3Univ. of North Carolina at Chapel Hill, Morehead City, NC, 4Univ. of Illinois at Chicago, Chicago, IL, 5USGS, Columbus, OH

1 Background
Current research conducted to inform new or revised water quality criteria has primarily focused on marine coastal and Great Lakes waters. A significant knowledge gap exists for how the data for marine and Great Lakes waters can be extrapolated to inland waters, including inland waterways, flowing rivers and streams, reservoirs, and lakes. Inland waters differ from coastal waters in terms of land use, hydrology and geology, sources of fecal contamination, and proximity to contamination sources. While culture-based methods only assess viable organisms, QPCR measures viable, culturable, stressed, dead organisms, and free nucleic acid. Potential persistence of any of these elements could lead to QPCR false positives (Lavender and Kinzelman 2009). Therefore, the background level of extracellular or dead cell DNA (ambient background DNA) must be taken into account when monitoring these aquatic environments. Successful implementation of molecular methods will also require the converse, inhibition of the QPCR reaction, to be addressed. Other issues such as precision, accuracy, relationship to health effects, and inter-laboratory variability will also impact successful implementation across water body types and Clean Water Act purposes.

2 Methods
Water samples were analyzed for a suite of EPA-approved FIB culture-based methods and, where possible, defined substrate technology culture-based methods. These results were compared with a suite of QPCR-based methods [for quantification of E. coli, Enterococci, and total Bacteroidales spp. (data not shown)].

Methods employed:
IDEXX Colilert-18™ and Enterolert™
EPA Method 1600 (Enterococci, mEI agar) & EPA Method 1603 (E. coli, modified mTEC agar)
Enterococci QPCR as per Haugland et al. and recent modifications in EPA Draft Method 1606
Bacteroidales spp. QPCR as per Siefring et al. (2008) and refined by Shanks et al. 2008
E. coli QPCR (Griffith et al. 2007 and as per Noble et al. 2009, submitted)

3 Results
E. coli was measured bi-monthly for a period of six months (2007 – 2008) using both QPCR and IDEXX ColiFert-18™ (Root River, Racine, WI) at sites chosen for their historic water quality, proximity to infrastructure, and land use characteristics. Average viable organism counts and their corresponding DNA calculated cell equivalents (CCE via ddCT) were determined. In all cases, CCE were typically higher than most probable number (MPN) values. Log differences between CCEs & MPNs ranged from 0.50 - 0.90. Correlation was better at some sites when dry weather preceded the sampling event (no rain for ~48 hours and up to 2 weeks) although this observation was not absolute.

A subset (n = 3) of the previously monitored Root River sampling sites were analyzed for E. coli concentration weekly from 7/2009 – 12/2009 (n = 65). Forty-two (65%) samples demonstrated equivalency between the culture-based method and CCE; 8 (12%) samples had CCE > MPN/100 ml, and 15 (23%) exhibited potential inhibition, i.e. CCE < MPN/100 ml.

In order to assess replication and inter-laboratory variability, sets of 285 filters archived from a study of recreational surface waters in the Chicago area (Dorevitch 2009), including inland waters, were distributed to the Racine Health Department lab (A) and 2 additional laboratories (B, C) (2010). Filters were analyzed by QPCR as replicates for Enterococci. Figure 1 (A & B) below demonstrates characteristic within lab correlation of replicate samples as CCE.

4 Conclusions
Contributions of extracellular or dead cell DNA (ambient background DNA) may result in a significant disparity in numerical match and/or regulatory action when monitoring coastal and inland aquatic environments. Interpretation of overestimation must occur in the context of human health risk to avoid excessive regulatory actions leading to water body impairments.

In certain aquatic environments inhibition of the QPCR reaction may occur. This was noticed in Racine with increased frequency of sampling. Determination of inhibition may vary within laboratories (between analysts), between laboratories, and as a function of probe/primer constructs. Standardization of protocols to deal with inhibition must occur prior to QPCR method implementation.

Analyses of data quality, such as replication, precision within and between laboratories, and across molecular targets is needed in addition to agreement between culture-based methods and QPCR as a function of water body type and pollutant source prior to implementation. Research to this effect is ongoing.

5 References

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