Enterococci qPCR: Potential Impacts to POTWs and Permit Compliance

Toni Glymph, Microbiologist III
Analytical Microbiology & Biomonitoring
Metropolitan Water Reclamation District of Greater Chicago

POTW Perspectives

- Water Quality Standards to Permit Process
- Implementation
- Practical Reality

Water Quality Standards to NPDES

- The process begins when a state or Indian tribe establishes water quality standards for a water body within its jurisdiction, as required by the Clean Water Act.
  - Designated Use
  - Water Quality Criterion
  - Policy of preventing degradation of the water body

Water Quality Standards to NPDES

- NPDES effluent limits are established to insure that the point source discharges of pollutants treat to a level that do not cause the waterbody to exceed the established water quality standard.

Water Quality Standards to NPDES

- Effluent monitoring allows the NPDES authority to assess compliance with the required controls.
- Next, there is some process to assess the waterbody to determine if it is attaining the established standards.

Water Quality Standards to NPDES

- The point of this monitoring is to evaluate the condition of the water body and to establish a cause-and-effect relationship.
- In order to establish a cause-and-effect relationship there must be congruency between the cause and the effect.

Water Quality Standards to NPDES

- In order to establish a cause-and-effect relationship the interpretation the genome equivalents for Enterococci must be accurately converted to the number of colony forming units.

Water Quality Standards to NPDES

- Any proposed criterion must measure the efficacy of the treatment process.
- The results of a WERF Final Report entitled, “Molecular Alternatives to Indicator and Pathogen Detection: Real-Time PCR”, indicated that the real time result is inadequate to represent the efficacy of sewage treatment.

Table 9-2. Comparison of *Enterococcus* Quantification by Real-time PCR and Culture Assay in Environmental Waters.

Sample	Real-time PCR (Genomic copies/100 ml)	Culture assay* (CFU or MPN/100 ml)
Sewage		
9/15/03		
Primary effluent–Plant I	3.5×10^5	8.1×10^5
Secondary effluent–Plant I	1.4×10^5	6.0×10^5
Secondary effluent–Plant II	1.7×10^5	3.2×10^4
9/30/03		
Primary effluent–Plant I	2.6×10^6	1.8×10^6
Secondary effluent–Plant I	3.5×10^5	1.4×10^5
Primary effluent–Plant II	1.6×10^5	1.7×10^5
Secondary effluent with chlorine–Plant II	6.9×10^5	0
10/1/03		
Primary effluent–Plant I	6.4×10^5	2.0×10^6
Secondary effluent–Plant I	2.1×10^5	2.3×10^4
Primary effluent–Plant II	8.1×10^5	4.2×10^5
Secondary effluent with chlorine–Plant II	5.4×10^5	0
Coastal Waters		
San Diego Creek	4.5×10^3	8.8×10^2
Mid Newport Bay	2.4×10^3	7.2
Lower Newport Bay	7.3×10^2	59
Newport Dunes (multiple sites n=4)	$(2.8 \pm 2.0) \times 10^2$	Below detection**
Newport Dunes (multiple sites n=27)	Below detection	Below detection

*All samples except Newport Dunes samples were determined by membrane filtration; Newport Dunes samples were determined using Idexx kit.

**Detection limit for real-time PCR is 5 CFU/reaction, for culturing assay is 10 MPN/100 ml

Implementation

- How can we establish a relationship between a source and any water quality impairment?
- What non-attainment of standards will cause an impairment?
- How will waste load allocations be established?

Implementation

- How do we compare the results of culture-based methods and the qPCR method?
- How does the levels of protection compare?
- How do we justify the “need” for a rapid method for wastewater effluents with the cost of implementation.

Implementation

- Training & Resources
- Public Acceptance

Practical Reality