Microbiological Monitoring Methods Efforts

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Background

In the last few years, a number of new microbiological methods have been published and made available to stakeholders for monitoring ambient waters and wastewaters.

As technology continues to evolve, EPA continues to work with stakeholders to look at ways to enhance environmental monitoring and address analytical gaps.

EPA continues to work with laboratories and commercial vendors through the alternate test procedure program to approve alternate methods for limited and nationwide use under the Clean Water Act (CWA).

EPA's Recent and Ongoing Micro Method Efforts

- Methods 1642 and 1643 for male-specific (MS2) and somatic coliphage
- Methods 1696 and 1697 for microbial source tracking (MST)
- Revision of the Microbiological ATP Protocol

Methods 1642 and 1643

- EPA worked with internal and external stakeholders to optimize and validate Methods 1642 and 1643
- Culture-based methods for the detection of male-specific (MS2) coliphage
- EPA leveraged *Method 1602: Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure* developed for use under the Groundwater Rule

Method 1642

- To address the need for larger sample volumes for ambient waters a dead end hollow fiber ultrafiltration (UF) procedure was coupled with EPA Method 1602
- Following concentration, samples are assayed using the Single Agar Layer (SAL) procedure
- Validated in fresh and marine waters, and advanced treatment wastewater

Method 1643

- Developed for samples with relatively high ambient concentrations of coliphage
- 100 mL sample volume is assayed using the SAL procedure
- Validated in secondary wastewater (no disinfection) samples
- Can also be used for fresh and marine waters with high background levels of coliphage

Methods 1696 and 1697

- In response to stakeholders' needs, EPA developed and validated two molecular-based methods for microbial source tracking (MST) to characterize fecal pollution in recreational waters
- Methods were validated in a single MLV study
- The methods characterize the level of human fecal pollution in recreational waters based on the detection and measurement of human-associated gene sequences

Methods 1696 and 1697 (cont.)

- Methods include multiple controls to identify any potential interferences
  - Sample processing control – identifies variability in sample processing efficiency
  - Internal amplification control – interference due to inhibition or competition
- Calculation Tool is available with the methods for use by stakeholders

Method 1696

- Measurement of human-associated gene sequences from Bacteroides
- HF183/BACR287 assay
- Validated in fresh and marine recreational waters
- Results are reported as $\log_{10}$ copies per reaction

Method 1697

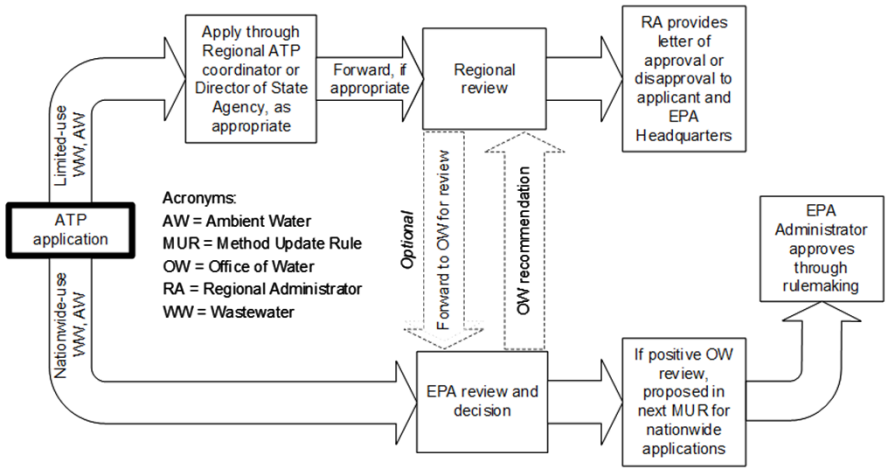
- Measurement of human-associated gene sequences from *Bacteroides-like microorganisms*
- HumM2 assay
- HumM2 qPCR Primer Set is patented and requires a license for use
- Validated in fresh and marine recreational waters

Current Method Efforts

- EPA is working with internal and external stakeholders to identify analytical needs
- Updating CWA and biosolids methods based on comments from laboratories
  - Adding clarification
  - Ensuring consistency between methods
  - Fixing typos
  - No procedural changes

Alternate Test Procedure Program

- Revising the Microbiological ATP protocol to provide clarifications
  - Based on questions from applicants
  - Related to the approval process and time frame



Methods Update Rule (MUR)

- The Proposed MUR includes
  - Updating Standard Methods 9221, 9222, 9223, and 9230 to the 23rd Edition in Tables IA and IH
  - Adding Method 1623.1 to Table IH
  - Adding the recently approved micro ATP KwikCount™ EC Medium for *E. coli* to Table IH
  - Differentiating between methods for biosolids and wastewater in Table IA