Performance-Based Method Systems (PBMS) Validation of a Method 1601 Same-Day Detection of Coliphage in Water

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Introduction

Coliphage is one of the fecal indicators that can be used in monitoring drinking water under the Ground Water Rule. Unlike bacterial indicators, coliphage viruses have greater survivability and may serve as better predictors of ground water quality. Simple coliphage methods are needed to indicate fecal and viral contamination of water.

Coliphage EPA Method 1601 is a performance-based method system (PBMS) where performance specifications were developed through collaborative testing and published as part of the method. Method improvements may be demonstrated using performance criteria that allow the modifications to be validated as an improved EPA method. This study describes Tier-1 multiple laboratory internal validations of a 16-hour modification of Method 1601 called Fast Phage™. Fast Phage features a same-day,<8 hour, fluorescent prediction step that was also evaluated in this project. This study was performed in advance of a proposed Tier-2 validation necessary for national approval of PBMS method modifications.

Table 1: Summary of Fast Phage Modifications Compared to Method 1601

METHOD 1601	FAST PHAGE MODIFICATION
Pre-culture *E. coli* host (CN-13 for somatic and F_{amp} for F+) overnight. Transfer to TSB and bring to OD 520_{nm} = 0.1 - 0.5. Ice.	Pre-dispensed dried media components in dissolvable film. Formulation proprietary for rapid amplification. Add STEP-1 media pouch to water sample in test vessel. Gently swirl to dissolve contents ~ 10 minutes.
Prepare 10x TSB, $MgCl_2$, nalidixic acid solutions in advance and autoclave.	
Add prepared solutions and *E. coli* to water sample in precise sequence of additions and temperatures.	Add *E. coli* tablet reagent (CN-13 for somatic and F_{amp} for F+). Swirl to dissolve tablet.
Incubate overnight at 36° ± 1°C air incubator.	Incubate 30 minutes in water bath at 38° ± 1°C. Incubate 4 hours and 30 minutes in air incubator at 39° ± 1°C.
Use pre-cultured log phase *E. coli* to make plaque plates. Prepare TS agar, autoclave and add pre-prepared and sterile filtered antibiotic solution after temperate.	Use *E. coli* tablet as TSB inoculum. Use supplied freeze-dried antibiotic (nalidixic acid for somatic or ampicillin-streptomycin for F+) to make solution. Prepare TS agar, autoclave and add antibiotic solution after temperate.
Transfer 10 ul of enrichment culture (24 hours) to plaque plate. Incubate overnight at 36° ±1 °C air incubator.	Transfer 10 ul of STEP-1 enrichment culture (5 hours) to plaque plate. Incubate overnight at 39° ± 1°C air incubator.
NO RAPID PREDICTION OF PLAQUE RESULT.	Transfer STEP-1 enrichment culture (1 ml for F+ or 10 ml somatic) to 100 ml STEP-2 indicating medium. Incubate up to 3 hours for fluorescence positive to predict positive plaque result.

Method

Fast Phage method modifications compared to Method 1601 are described in Table 1.

Laboratories obtained their own wastewater, reagent water and ground water samples. Charm Sciences supplied funding, kits for somatic and male-specific coliphage and disposable supplies.

Coliphage in wastewater effluents were quantified in plaque forming units per milliliter (pfu/ml) using Double Layer Agar Technique as described in Method 1601.

Wastewater was diluted in 1.1 L water samples and mixed for 20 minutes to achieve a pfu/100 ml concentration near the EPA Method 1601 performance criteria level: 1.5 pfu/100 ml with somatic coliphage and 1.3 pfu/100 ml with male-specific coliphage.

Ten replicate 100 ml samples of each water type were tested with Fast Phage Method 1601 modification which consists of a 5-hour STEP-1 enrichment followed by an overnight plaque determination, STEP-1 plaque.

The laboratories also transferred the STEP-1 enrichment into a same-day fluorescent predictor of coliphage presence, STEP-2 fluorescence. The transfer aliquot into fluorescent media incubated 3 additional hours for a total assay time of 8 hours. Laboratories also performed an overnight plaque determination at the end of the fluorescent prediction inoculation, STEP-2 plaque.

Table 2: Fast Phage Somatic Presence/Absence Testing in 5 Laboratories

	pfu/100 ml Spike Level	Reagent Water Fluorescent Positives	Reagent Water STEP-1 Plaque	Reagent Water STEP-2 Plaque	Ground Water Fluorescent Positives	Ground Water STEP-1 Plaque	Ground Water STEP-2 Plaque
Lab 1	1.5	10	10	10	10	10	10
Lab 2	1.5	7	7	7	8	8	9
Lab 3-1	1.7	9	10	10	10	9	9
Lab 3-2	1.5	10	10	10	10	10	10
Lab 4-1	1.7	7	7	9	9	8	9
Lab 4-2	1.4	7	7	7	5	6	6
Lab 5	1.5	10	7	10	10	6	10

Results

All five laboratories successfully meet EPA Tier-1 performance criteria by detecting at least 50% STEP-1 plaque positive samples from spiked reagent and ground water when coliphage are at the Method 1601 specified pfu/100 ml or lower, as shown in Tables 2 and 3.

The results of the fluorescence prediction were correlated to the STEP-1 and STEP-2 plaques in four-fold table comparisons. The somatic same-day fluorescent prediction is 92.1% predictive of STEP-1 plaque formation with a 1.7% false-negative and a 7.4% false-positive rate (Table 4). Plaque results of STEP-2 show that 8 of 9 false-positive fluorescent results reported in Table 4 were true positives, indicating a true fluorescence false-positive rate of 0.8%.

The male-specific fluorescent prediction is 83.6% predictive of STEP-1 plaque formation with a 14.7% false-negative rate and a 6.2% false-positive rate (Table 5). Plaque results of STEP-2 show that 7 of 8 false-positive fluorescent results reported in Table 5 were true positives, indicating a true fluorescence false-positive rate of 0.8%. Fewer fluorescent false-negatives (8 of 117 samples) were observed with labs achieving the target spike level of 1.3 pfu/100 ml compared to labs with spike levels lower than ~1.1 pfu/100 ml (15 of 60 samples), indicating better fluorescence prediction at higher plaque levels.

Table 3: Fast Phage Male-Specific Presence/Absence Testing in 5 Laboratories

	pfu/100 ml Spike Level	Reagent Water Fluorescent Positives	Reagent Water STEP-1 Plaque	Reagent Water STEP-2 Plaque	Ground Water Fluorescent Positives	Ground Water STEP-1 Plaque	Ground Water STEP-2 Plaque
Lab 1-1	1.11	7	8	8	4	8	8
Lab 1-2	1.27	9	9	9	8	8	9
Lab 2-1	0.88	2	6	8	1	5	7
Lab 2-2	1.45	3	4	6	7	9	10
Lab 2-3	1.24	5	5	5	9	9	9
Lab 3-1	1.32	10	10	10	10	10	10
Lab 4-1	1.1	9	10	10	10	10	10
Lab 4-2	1.3	10	10	10	10	10	10
Lab 5	1.3	7	5	10	9	6	8

Laboratory Participants

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Summary

Five laboratories demonstrate they meet Method 1601 PBMS Tier-1 criteria with Fast Phage enrichment and plaque assays. Therefore, they may perform Fast Phage as a qualified modification of Method 1601 for potable ground water.

The Fast Phage modification accelerates Method 1601 total-time-to-result to less than 24 hours using convenient, ready-to-use reagents.

The same-day fluorescent prediction is valuable as a rapid fecal indicator with a low 1% false-positive rate. This allows early detection and remediation with positive samples as well as early prediction of remediation effectiveness.

A negative fluorescent prediction, particularly with male-specific coliphage, should wait for plaque absence as confirmation of result. This is consistent with Fast Phage instructions.

The Fast Phage modification meets the EPA requirements for national Tier-2 PBMS validation which specify 75% of participating laboratories meet Method 1601 performance criteria with shared effluent for spiking.

Table 4: Somatic Coliphage Fluorescent Prediction Compared to STEP-1 Plaque

	STEP-1 Plaque		
STEP-2 Fluorescence	Positive	Negative	Total
Positive	113	9	122
Negative	2	16	18
Total	115	25	140

Sensitivity 98.3% False-Negative Error 1.7%
Specificity 64.0% False-Positive Error 7.4%
Overall Agreement 92.1%

Table 5: Male-Specific Coliphage Fluorescent Prediction Compared to STEP-1 Plaque

	STEP-1 Plaque		
STEP-2 Fluorescence	Positive	Negative	Total
Positive	122	8	130
Negative	21	26	47
Total	143	34	177

Sensitivity 85.3% False-Negative Error 14.7%
Specificity 76.5% False-Positive Error 6.2%
Overall Agreement 83.6%