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Same Day and Continuous Detection of Coliphage in Water

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Fast Phage™

Purpose

The purpose of this study is to simplify coliphage detection to meet drinking water standards within one working day. Described are a presence-absence (PA) method for 100 ml samples, MPN quantitation techniques for 1-100 ml samples, and a continuous detection technique over 24 hours, testing liter volumes.

Background

Recently, coliphage – viruses to the coliform group of bacteria – were listed as an equivalent fecal marker to *E. coli* in the Ground Water Rule (GWR). Half of waterborne illnesses are viral in origin. Public health officials agree that bacterial indicators and viral indicators provide valuable information about water sources and processes. Comprehensive risk assessment of water should include tests for both bacterial and viral indicators. Conventional coliphage detection methods have been difficult, time consuming and expensive. Bacterial indicator tests have been used predominantly because of ease of use and low cost. Simple, fast and low-cost coliphage methods are needed to compliment bacterial testing.

Materials and Methods

PA Method: Modifications to Method 1601, called Fast Phage™, are summarized in Table 1. Wastewater-spiked water samples at 0.5 pfu/100 ml were split into 20 replicates and tested by Method 1601 and Fast Phage. After enrichment step (5hrs) a transfer to MUG-gal media predicts by fluorescence (366 nm) the next-day plaque results in less than 3 hours (total time from assay start <8 hours). A variety of secondary wastewaters pre-and post-chlorine disinfection were used to spike ground water.

MPN Methods – TEMPO® Method: 1 to 3.7 ml water sample plus 300 ul of 5 ml saline rehydrated CN-13 *E. coli* tablet to final 4 ml volume are added to TEMPO® card.
100 ml Method: 1-95 ml sample to final 95 ml volume and add STEP-2 pouch. Sample is pre-heated in 38°C water bath while *E. coli* tablet is rehydrated for 10 minutes in 5 ml saline. Contents are combined and sample divided. In this experiment, Quanti-Tray® MPN are performed. Comparative **Methods 1601, 1602 or Double Layer Agar** are also performed on same samples. MPN devices are incubated 5.5 - 6 hours. MPN are determined from fluorescent (366 nm) sub-divisions and using MPN table.

Continuous Method: A continuous monitor of coliphage was constructed, **Figure 1**, by culturing *E. coli* host in a continuously fed water sample/media mix in a disposable container with external monitoring probes. Transmittance 520 nm 20 cm path length T=0.25 is measured by a probe/reflector. Software monitors transmittance and activates peristaltic pump that in-feeds mix and out-feeds *E. coli* culture to maintain constant T. Fluorescence 366 nm (450 nm excite) is monitored by Turner Designs C-7 probe. 1.0 mV activates warning of coliphage host infection. A mix pump combines sample with 10x media concentrate and a 90°C heated coil is used for culture disposal.

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

METHOD 1601	FAST PHAGE MODIFICATION
Pre-culture *E. coli* CN-13 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. Film pouch also contains *E. coli* tablet (CN-13 for somatic and F_{amp} for F+) inoculum that dissolves.
Add prepared solutions and *E. coli* to water sample in precise sequence of additions and temperatures.	Add media/*E. coli* pouch to water sample in test vessel. Gently swirl to dissolve contents ~ 10 minutes.
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 (naladixic 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 enrichment culture (5 hours) to plaque plate. Incubate overnight at 39° ± 1°C air incubator.
NO RAPID PREDICTION OF PLAQUE RESULT.	Transfer enrichment culture (1 ml for F+ or 10 ml somatic) to 100 ml indicating medium STEP-2. Incubate up to 3 hours for fluorescence positive to predict positive plaque result.

Figure 1: Continuous Testing System

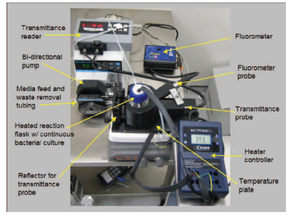

Figure 1 Legend: 60 - 150 ml bacterial culture in a disposable flask in probe holder on a heated base plate at 38°C. Media/water-sample mix is fed in and culture removed at the same rate using disposable tubing and external transmittance probe-reflector with computer software (not shown). External fluorometer probe detects coliphage infection of culture.

Results & Discussion

Table 2 compares positives of 20 replicates detected by Method 1601 to those detected by Fast Phage STEP-1 plaque positive and fluorescence.

Table 3 relates the accuracy of the fluorescent indicator at predicting the STEP-1 plaque results. The predictive correlation is 90%. Disinfected waters had 7 false negatives for fluorescence, while untreated waters had 5 fluorescence positives without STEP-1 plaque confirmation, but with detected coliphage from STEP-2 plaque.

Table 4 reports somatic phage TEMPO MPN determinations of non-disinfected secondary waste waters compared to Method 1602 of plaque forming units (pfu). **Table 5** reports somatic phage by 100 ml MPN technique. Results are in agreement within 0.5 log. CVs of replicate MPN are about 30 - 50%.

Figure 2 demonstrates a continuous overnight sampling of over 10 L water with a next morning challenge of 20 pfu F+ coliphage in 1 L using F_{amp} *E. coli* host. Positive fluorescence is 5.5 hours post-phage challenge. Plaque were confirmed from fluorescent positive sample. Similar results using CN-13 host are observed with a daily flow rate of about 4-6 liters/day. No fluorescence is observed in unchallenged water samples with up to 20 L sample passing through the culture flask over the 24 - 48 hour sampling period. Biofouling at 24 - 48 hours requires disposal and exchange of flask/tubing.

Table2: Comparability Study

Waste Location	Chlorine Disinfected	Dilution	EPA 1601 Plaque	STEP-1 Plaque	Fluorescence Positive	Total Samples
M 10-15-08	no	2.25 ml / 4.5 L	15	15	16	20
L 09-09-08	no	4.5 ml / 4.5 L	12	10	11	20
SA 12-18-08	no	0.35 ml / 4.5 L	10	6	7	20
L 06-26-08	yes	10 ml / 4.5 L	15	13	7	20
O 12-30-08	no	1.9 ml / 4.5 L	10	9	11	20
O 01-02-09	yes	280 ml / 4220 L	5	4	3	20
Sum of Positives			67	57	55	120
% Positive			55.8%	47.5%	45.8%	

Table 3: Fluorescence Prediction of STEP-1 Plaque Results

Fluorescence	STEP-1 Plaque		Total F
	Positive	Negative	
Positive	50	5	55
Negative	7	58	65
Total STEP-1	57	63	120

	Percent
Sensitivity	87.7%
Specificity	92.1%
False Positive Error	9.1%
False Negative Error	12.3%
Overall Agreement	90.0%

Table 4: MPN Determination of Somatic Phage in ml of Secondary Wastewaters Compared to 1602 Plaque Determination

Secondary Wastewater	Method 1602		TEMPO	
Sample 1 ml/test	Average (N=4)	Stdev	Average (N=6)	Stdev
M 1015	52	13	42	18
L 0909	12	4	10	4
I 0623	34	8	39	13
I 0623 1:5	6.5	2	6	2
O 1230	16	3	14	8
V 1223	56	8	65	41
L 012209	526	56	197	62
x174/ml PC1118	77	19	49	19
x174/bottle PC 1111	173	11	101	28

Table 5: MPN Determination of Somatic Phage in 1 ml of Secondary Wastewaters Compared to Double Layer Agar and Method 1601 MPN

Waste Water	Quanti-Tray MPN/ml Average N=2	Double Layer pfu/ml	Enumeration MPN 1601 pfu/ml
L 01-23-08	163.6	107	66.7
L 01-23-08 1:10	14.5	10.7	6.7
W 02-01-08	4.1	12.5	4.5
M 01-15-08	26.7	40	10

Figure 2a: Transmittance Signal of F_{amp} Continuous Run

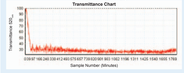

Figure 2b: Fluorescent Signal of F_{amp} Continuous Run

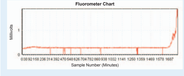

Figure 2c: Flow Rate of F_{amp} Continuous Run

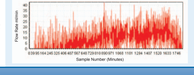

Figure 2a Legend:
60 ml culture of *E. coli* F_{amp} was grown to transmittance 0.25 at which time media mixed with water sample was fed into the system. 10 L of clean water were sampled overnight. The next morning (sample 1280) 20 pfu F+ coliphage in 1 liter was introduced into the system. Then uncontaminated water was reconnected.

Figure 2b Legend:
Fluorescent reading of the *E. coli* during their growth and continuous state. Phage infection starts to be detected at sample (minute) 1687 and by sample 1770 fluorescence is 1.0 mV where a warning signal trips that infection has occurred.

Figure 2c Legend:
Flow rate is 0 ml/min until transmittance meets target of 0.25 when pump is activated. Rate is determined by software algorithm that has a high-low flow inflection at the target transmission. As transmission gets less than target, flow jumps. When transmission is higher than target, flow rate slows.

Summary

Fecal assessment by detecting less than 1 pfu coliphage in 100 ml water meets ground water detection guidelines and can now be done in less than 8 hours.

Fast Phage 100 ml P/A modifications simplify Method 1601 to a 16-hour procedure with an 8-hour 90% correlative fluorescent prediction.

Somatic MPN techniques offer 6-hour fecal indicator assessment of fresh, untreated source water, providing same-day pollution-risk assessment.

Continuous sample monitoring for coliphage is achievable with 4-6 hour detection. This offers liter volume testing of continuously sampled water as well as automation of fecal risk assessment.

References

1. National Primary Drinking Water Regulations: Ground Water Rule [Federal Register: November 21, 2006 (Volume 71, Number 224)]
2. Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure, EPA Number 821R1030 April, 2001
3. Method 1602 Male-Specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure, EPA Number 821R1029 April, 2001