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Project Summary

Sampling Regimes and Bacteriological Tests for Coliform Detection in Groundwater

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Since conventional procedures can fail to detect coliforms in potable water, the effects of increased sampling frequency and alternative bacteriological media on coliform detection were evaluated for samples from groundwater-fed public water supply systems. For 1,560 drinking water samples collected from 10 small water systems in western Oregon, the presence-absence (P-A) test detected significantly more coliform-positive samples than either the conventional membrane filtration (MF) test using mEndo agar-LES or the 5- and 10-replicate fermentation tube (FT) test. No difference was found in coliform detection based on five samples collected on the same day or throughout the sampling interval at either the same or different locations. A fivefold increase in sampling frequency increased the incidence of coliform detection 2.9- to 5.0-fold.

An additional study of 600 water samples demonstrated that 5 alternative coliform tests, including the presence-absence test and 4 MF media formulations (m-T7, mTECmod, HABmod, and mLS) detected significantly more coliform-positive samples than the conventional MF test under either aerobic or anaerobic conditions. Coliform densities from the m-T7 test were 1.4 to 2.1 times greater than for the other MF tests.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, Ohio, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

The routine monitoring of coliform bacteria in water supplies involves two critical steps. First, samples must be collected in a manner that allows a sufficiently accurate assessment of coliform occurrence in the distribution system. Increases in sampling frequency for small water supplies beyond the current once-per-month requirement raise the possibility of designing sampling regimes based on spatial or temporal patterns or both. Second, since conventional coliform detection procedures can fail to detect coliforms from an often stressful environment, alternative media and incubation procedures must be evaluated.

The two objectives of this investigation were to determine if a fivefold increase in sampling using three alternative sampling regimes would significantly increase coliform detection, and to compare coliform detection by the conventional aerobic membrane filtration test using mEndo agar-LES (STD-MF) with the presence-absence test (P-A) and five alternative MF procedures. The MF alternatives were an agar medium (mTECmod) using lauryl sulfate broth (mLS), m-T7 medium, modifications of the mTEC (mTECmod) medium for detection of *E. coli,* an experimental carrageenan-containing medium (HABmod), and anaerobic incubation of mEndo agar-LES (AN-MF).

Materials and Methods

Initially, 1,560 water samples were collected in the Willamette River Valley of western Oregon from 10 small public water supply systems that serve less than 1,000 people. Sampling was based on 5-week intervals, using five locations

per water system, to evaluate sampling from different locations at the same time or from either the same or different locations throughout the sampling interval. At the end of this study, an additional 288 samples were collected weekly at 3 locations within 8 water systems to generate a total of 600 samples for the alternative media study.

For the sampling frequency study, 100-mL subsamples were tested for the presence of coliforms using a 10-replicate fermentation tube (FT10) procedure, a membrane filtration test using mEndo agar-LES (STD-MF), and a presence-absence (P-A) test as recommended by Standard Methods for the Examination of Water and Wastewater (16th Ed., American Public Health Association, 1985). For the alternative media study, additional 100-mL subsamples were filtered and incubated aerobically on HABmod, mLS, mTECmod, and m-T7 media. A second mEndo agar-LES plate was incubated under anaerobic conditions (AN-MF).

After 24 and 48 hours at 35°C, all gas-positive FT tubes and all acid or acid+gas P-A bottles were inoculated into 2% brilliant green bile broth (BGB) to confirm the presence of coliforms by gas production with 48 hours. For the MF tests, after 22 hours incubation for the STD-MF, HABmod, mLS, mTECmod, and m-T7 tests and 48 hours for the AN-MF test, typical colonies were counted and verified as coliforms by gas production, first in lauryl tryptose broth (LTB) and then in BGB.

Significant differences in coliform detection by the different sampling regimes and bacteriological tests were evaluated using the chi-square statistic (McNemar's test). Differences in coliform density estimated by the MF tests were evaluated using paired t-tests of the coliform-positive samples. The goodness-of-fit between the observed coliform frequencies and either the negative binomial or Poisson distribution was evaluated using the chi-square statistic.

Experimental Results

Sampling Frequency Study

Analysis of 1,560 samples resulted in 352 coliform-positive samples. The incidence of positive samples by bacteriological test and the results of 2,382 individual tests (FT tube, P-A bottle, or MF colony) are compiled in Table 1. The P-A test significantly outperformed the FT10, which in turn outperformed both the STDMF and FT5 tests. Additional breakdown of coliform-positive samples showed 27.8% were detected by all three tests, 14.2% by FT and P-A, 8.5% by STD-MF and P-A, 4.5% by FT, and STD-MF, 23% by P-A, 13.4% by FT and 8.5% by STD-MF.

The confirmation or verification efficiencies were highest for the FT test (77%), intermediate for total P-A results (62.9%), and lowest for the STD-MF (47.4%). The overall efficiency of the P-A test was strongly influenced by the very low confirmation rate (4.2%) of the 142 acid-only presumptive P-A bottles.

After partitioning the coliform-positive samples into the original four sampling regimes, our data (Table 2) showed that a fivefold increase in sampling frequency resulted in a 2.9- to 5.0-fold increase in coliform detection over the once-per-interval estimate, depending on the sampling regime and bacteriological test used. For any one test there was no significantly superior sampling regime although there were some significant differences between tests within a given sampling regime.

Alternative Media Study

The incidence of positive samples and population characteristics of 600 water samples processed by 7 bacteriological procedures are listed in Table 3. The greatest number of coliform-positive samples was detected using the m-T7 test, although the P-A results were not significantly lower. The m-T7 results were also significantly higher than those produced by the mTECmod, HABmod, or mLS tests; which in turn were significantly higher than the results on mEndo agar-LES under either aerobic or anaerobic incubations.

The statistics describing the coliform frequency distributions also varied with the MF test employed. The observed distributions for all six MF procedures were significantly different from a Poisson distribution. All distributions, except for HABmod, were adequately explained by a negative binomial function. The parameters defining the negative binomial distribution — mean and coefficient of aggregation — changed substantially with the different procedures.

Paired comparisons showed the m-T7 to produce coliform density estimates 1.4- to 2.1-fold greater than the other MF tests with both the m-T7 and mTECmod results being significantly higher than the STD-MF results.

Conclusions

The results of this project indicate that: the currently accepted replicate fermentation tube test and membrane filtration test using mEndo agar-LES are inadequate in detecting either the incidence or densities of coliforms in groundwater; increased sampling frequency increases

Table 1. *Incidence of Coliform Positive Samples and Results of Individual Tests.*

	FT5	FT10	MF	P-A
Positive Samples	*165[a]*	*211[b]*	*174[a]*	*259[c]*
Presumptive Positive Tests	—	*795*	*1175*	*142 (acid) 270 (acid+gas)*
*Efficiency**	—	*77.0%*	*47.4%*	*4.2% (acid) 93.3% (acid+gas)*

[a,b,c] - values followed by different letters were significantly different ($p<0.05$), by McNemar's Test
** - confirmation or verification efficiency*

Table 2. *Incidence of Coliform Detection by Four Sampling Regimes.*

		5 Samples on Same Day	5 Samples throughout 5-wk Interval	
Test	*Single Sample*	*Different Sites*	*Same Site*	*Different Sites*
FT10	*19*	*84*	*79*	*78*
STD-MF	*20*	*58**	*77*	*69*
P-A	*20*	*99*	*100*	*100**

** - column (test) results were significantly different from others*

Table 3. *Incidence of Positive Samples and Population Characteristics of 600 Water Samples Processed by 7 Bacteriological Procedures.*

		Membrane Filtration Procedures					
		mEndo agar-LES					
Statistics	*P-A*	*Anaerobic (AN-MF)*	*Aerobic (STD-MF)*	*mTECmod*	*HABmod*	*mLS*	*m-T7*
Positive Samples	$109^{c,d}$	58^{a}	64^{a}	102^{c}	$91^{b,c}$	79^{b}	121^{d}
Mean	*na*	*0.48*	*0.74*	*1.00*	*0.87*	*0.65*	*1.48*
Variance	*na*	*14.8*	*34.0*	*32.2*	*31.7*	*17.0*	*59.2*
*K**	*na*	*0.0396*	*0.0362*	*0.0638*	*0.0549*	*0.0543*	*0.0686*

^{a,b,c,d} - *values followed by different letters were significantly different ($p<0.05$) by McNemar's Test*
na - not applicable to P-A test
** - coefficient of aggregation*

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The complete report, entitled "Sampling Regimes and Bacteriological Tests for Coliform Detection in Groundwater," (Order No. PB 88-107 230/AS; Cost: $11.95, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
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coliform detection, although there is no apparently superior strategy for timing or location of sample collection, and selection of a specific mathematical model used to describe the distribution of coliforms in groundwater is dependent on the bacteriological test used in the initial observations.

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