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TECHBRIEF
Environmental Technology Verification Program

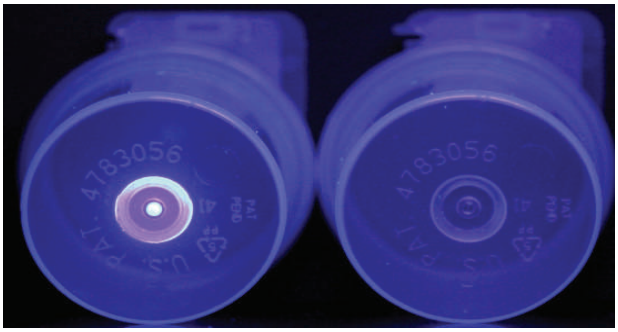
ETV

E. coli and Total Coliform Detection in Drinking Water

Human waterborne diseases often result from exposure to water that is contaminated with the feces of warm-blooded animals, including humans. Public water systems (PWS) are required to produce and distribute drinking water that is free from fecal contamination. A Federal regulation, the Total Coliform Rule (TCR), requires PWS to monitor for the presence of specific bacterial organisms that serve as indicators of fecal contamination. These indicator organisms are total coliforms (TC) and *Escherichia coli* (*E. coli*). In 2010 and 2011, the U.S. EPA Environmental Technology Verification (ETV) Program's Advanced Monitoring Systems (AMS) Center, operated by Battelle under a cooperative agreement with EPA, evaluated the performance of two detection technologies for TC and *E. coli*. Both technologies were designed to detect the presence or absence of TC and *E. coli* in water. Technology descriptions are provided in Table 1.

Technology Description and Verification Testing

The TCR specifies the approved methods to detect these indicator organisms in water. The TCR also allows for entities to apply for Agency permission to use an alternate test procedure in place of an EPA-approved method. The Alternate Testing Procedures (ATP) program uses a protocol in which 200 water samples, roughly half of which are positive and half are negative, are analyzed with a reference method and the proposed new method. Such a protocol design is considered adequate for evaluation of methods proposed for low-level organism detection. Due to constraints in the number of samples that could be generated for this study, a modified ATP protocol was used in which raw sewage was collected and chlorinated to achieve a 2-4 log reduction in bacterial concentration. The resulting chlorine-stressed bacteria were then diluted with de-chlorinated tap water to achieve low organism density in the fractional positive range to spike drinking water dilution sets that yielded a 50 ± 25% positive result for both the TC and *E. coli*.



Positive (left) and negative (right) results of an ETV-verified technology.

Standard Method (SM) 9221B for TC and SM9221F for *E. coli* were the reference methods of choice used for domestic verification. One vendor also chose to have *E. coli* results compared to the Colilert®-18 reference method for possible verification in Denmark. The verified detection technologies utilize fluorogenic enzyme substrate compounds to detect the presence of enzymes specific to TC and *E. coli*.

Environmental and Public Health Regulatory Background of Total Coliform and *E. coli* at a Glance

Total Coliform bacteria (TC) are a group of bacteria that are regularly present in environmental waters. Fecal coliforms (FC) and *E. coli* are a sub-group of TC that are more associated with the feces of people and warm-blooded animals. FC or *E. coli* presence can indicate contamination of water supplies resulting in an increased risk of the presence of waterborne pathogens. Bacterial indicators such as TC and *E. coli* are also valuable indicators of the performance of drinking water treatment processes and distribution system integrity.

On June 29, 1989, U.S. EPA issued the Total Coliform Rule (TCR) which sets both maximum contaminant limit goals (MCLGs) and maximum contaminant limits (MCLs) for TC and *E. coli* in drinking water (DW) supplies. The TCR requires all public water systems (PWS) to monitor for the presence of TC and *E. coli* in the distribution system. Distribution systems must collect and test samples on a monthly basis, with no more than 5% of those DW samples being TC-positive. If a sample result is TC-positive, then the sample must be further tested for the presence of FC or *E. coli*. The distribution system population and/or the number of service connections in the system serve as the basis for the number of samples tested per month.

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[1]The ETV Program operates largely as a public-private partnership through competitive cooperative agreements with non-profit research institutes. The program provides objective quality-assured data on the performance of commercial-ready technologies. Verification does not imply product approval or effectiveness. EPA does not endorse the purchase or sale of any products and services mentioned in this document.

Table 1. Description of Total Coliform and *E. coli* Detection Technologies		
Vendor and Technology Name	**Type of Result**	**Technology Description**
Colifast *ALARM At-Line Automated Remote Monitor*	Presence/ absence	The technology is a system that uses the substrate 4-methylumbelliferyl (MU)-β-D-galactoside that is hydrolyzed by the β-D-galactosidase enzyme for TC detection and the substrate 4-MU-β-D-glucuronide that is hydrolyzed by β-D-glucuronidase enzyme for *E. coli* detection. An increase in the amount of TC or *E. coli* leads to an increase in the fluorescent product MU, which is detected at specific wavelengths by an internal spectrophotometer. Can be operated in both manual or continuous detection modes.
Pathogen Detection Systems (PDS) *Automated Microbiology Platform (AMP)*	Presence/ absence	The technology is a bench top incubator/analyzer/data logger system that utilizes an enzyme substrate (β-galactosidase enzyme for TC and β-glucuronidase enzyme for *E. coli*) to detect the presence of TC and *E. coli*. Enzymes produced by TC and *E. coli* cleave the substrate, resulting in the release of fluorescent products. These products accumulate in an optical sensor detected by an ultraviolet light source/charge-coupled device optical detection system. Can be operated in both manual or continuous modes.

ETV AMS initiated verification testing in the summer of 2010 when raw sewage samples were taken from Southerly Wastewater Treatment Plant (SWTP) in Columbus, Ohio. Technicians evaluated a total of 20 samples by comparing the reference method proportion of positive and negative results to resulting proportions produced by the tested technologies. From this comparison, the false positive (specificity) and false negative (sensitivity) rates were calculated. Technologies were also evaluated for operational factors such as ease of use, analysis time, laboratory space and equipment required. Table 2 provides selected performance data for the two ETV-verified detection technologies. Additional information is available in the verification reports and statements on the ETV website at http://www.epa.gov/nrmrl/std/etv/vt-ams.html#ecoli.

Potential Outcomes of Verified Total Coliform and *E. coli* Detection Technologies

Verification results show that these two detection technologies can detect both TC and *E. coli* in water samples. Operators can run both of these technologies in an automated or a continuous detection mode following sample setup, obtaining results in as little as 14 to 24 hours compared to the conventional standard methods that range from 48 to 72 hours.

Table 2. Selected Verification Results for Total Coliform and *E. coli* Detection Technologies					
Technology Company[1]	Organism	Sensitivity[3]	Specificity[4]	False Positive Rate	False Negative Rate
Colifast	TC	100%	100%	0%	0%
PDS 18h[2]	TC	65%	100%	0%	35%
PDS 24h	TC	91%	82%	18%	9%
Colifast	*E. coli*	75%	100%	0%	25%
PDS 18h	*E. coli*	67%	100%	0%	33%
PDS 24h	*E. coli*	67%	100%	0%	33%

1. Sewage samples were collected, chlorine-stressed, and spiked to a 10 organism/100mL dilution, except the Colifast test for *E. coli* used a 50 org/100mL dilution.
2. For the PDS TC sample, a dilution of 1 org/100mL also resulted in the targeted 50 +- 25% ratio but is not included in this table.
3. Sensitivity is defined as the percent of positive samples correctly identified as positive.
4. Specificity is defined as the percent of negative samples correctly identified as negative.

References:

Total Coliform Rule, United States Federal Register, 54 FR 27544-27568, June 29, 1989, Vol.54, No. 124

Revisions to the Total Coliform Rule, Proposed Rule, United States Federal Register, 75 FR 40926-41016, July 14, 2010 Vol. 75 No. 134

U.S. EPA Office of Ground Water and Drinking Water, http://water.epa.gov/drink/index.cfm

U.S. EPA ETV Program, http://www.epa.gov/etv

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