THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Verification Statement

TECHNOLOGY TYPE:	**COLIFORM DETECTION**
APPLICATION:	**ANALYSIS OF TOTAL COLIFORMS AND *E. COLI* IN DRINKING WATER**
TECHNOLOGY NAME:	**Colifast ALARM AT-LINE AUTOMATED REMOTE MONITOR**
COMPANY:	**Colifast**
ADDRESS:	**Strandveien 33, 1366 Lysaker Norway**
PHONE:	**+47 67 10 05 10**
WEB SITE:	**www.colifast.no**
E-MAIL:	**post@colifast.no**

The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field and laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six verification centers under ETV, is operated by Battelle in cooperation with EPA's National Risk Management Research Laboratory. The AMS Center evaluated the performance of a system for coliform detection in drinking water (DW). This verification statement provides a summary of the test results for ALARM At-Line Automated Remote Monitor by Colifast (Colifast ALARM).

VERIFICATION TEST DESCRIPTION

The U.S. EPA's 1989 Total Coliform Rule (TCR) sets both goals and legal limits for the presence of total coliform (TC) and *Escherichia coli* (EC) in DW. To summarize, the TCR states that the objective for water utilities is for zero TC organisms to be present in DW samples, and no more than 5% of all DW samples collected by a utility can test positive for TC. In order to comply with the TCR, water utilities need coliform detection technologies that are able to detect TC and EC at concentrations of one organism (org) per 100 milliliters (mL) of DW. While it is difficult to determine if a single target organism is present in 100 mL of water, when approximately half of the analyzed replicates are positive and half are negative, the density of the organism has become adequately low so that a positive result can be considered single organism detection. Therefore, for the purpose of this verification, the objective was to prepare a series of spiked DW dilution sets (labeled A, B, C) that provided 50 ±25% positive results for TC or EC (depending on which is being targeted) using the reference method(s), and compare the results from the reference method with those produced by the tested detection technology.

Results from the Colifast ALARM were compared to the results obtained from the reference methods American Public Health Association, American Water Works Association, and Water Environment Federation Standard Method (SM) 9221B (TC) and SM 9221F (EC) analyses that are presence/absence methods for TC and EC. In addition, this verification testing was also conducted in cooperation with ETV programs in Canada (ETV Canada) and Denmark (DANETV) as a possible ETV verification by those programs. In Europe, the Colilert-18 combined with Quanti Tray for quantification has been proven equivalent with ISO 9301-1, the accepted regulatory method in Europe. Colilert-18 is considered a relevant reference method (since it is the presence/absence version of the identical test) and was considered adequate to meet the DANETV requirement of use of a European accepted reference method to potentially grant DANETV verification following this test. Each of these reference methods utilize selective and/or chromatogenic liquid growth media to detect TC and EC. The verification test of the Colifast ALARM was conducted from August 31 through September 7, 2010 at Battelle in Columbus, Ohio with the reference method analyses being performed at Superior Laboratories in Galloway, Ohio. Technology operation and sample handling and analysis were performed according to the vendor's instructions. Both reference method and Colifast ALARM sample analysis results were reported as presence/absence.

Sample analysis results from the Colifast ALARM were evaluated by comparing the proportion of positive and negative results to the proportion of positive and negative results produced by the reference methods, including the comparison of false positive (FP) rate (or specificity) and false negative (FN) rate (or sensitivity). In addition, operational factors such as ease of use, required reagents, analysis time, and laboratory space and utilities required were reported.

QA oversight of verification testing was provided by Battelle and EPA. Battelle and EPA QA staff conducted technical systems audits of the testing and Battelle QA staff conducted a data quality audit of at least 25% of the test data. This verification statement, the full report on which it is based, and the test/QA plan for this verification test are available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The Colifast ALARM is an automated system for detection of TC or EC in 100 mL water samples. The Colifast ALARM automatically collects the water sample at programmed intervals for the analysis of TC and EC. The Colifast ALARM method is based on an enzymatic reaction. The Colifast TC medium contains the substrate 4-methylumbelliferyl (MU)-β-D-galactoside, and this substrate is hydrolyzed by the enzyme β-galactosidase that is present in TC. The Colifast EC medium contains the substrate 4-methylumbelliferyl (MU)-β-D-glucuronide, and this substrate is hydrolyzed by the enzyme β-D-glucuronidase that is present in EC. The fluorescent product MU is produced as a result of the hydrolysis reaction. The media contains inhibitors to hinder growth of non-coliforms.

A 100 mL water sample is added to a sample bottle and then incubated in and analyzed by the Colifast ALARM. The main components of the Colifast ALARM are the incubator reaction chamber, a flow

injection pump system for liquid handling and a detector system, including wavelength specific emitters combined with a spectrometer. The bacterial detection results are based on measured concentrations of the fluorescent product. An increase in the number of EC means an increase in the amount of β-D-glucuronidase (enzyme). This leads to an increase in the production of MU (the fluorescent product) that yields a higher fluorescence signal on the Colifast ALARM.

When the Colifast ALARM is operated in "at-line" mode, 100 mL samples are collected automatically using the pump system. The analysis is peformed automatically, and the software within the Colifast ALARM automatically interprets the fluorescent measurements hourly throughout the incubation time. A positive result is reported on the screen when the presence of TC or EC is detected, regardless of the amount of time that has passed. The results are stored on the system computer embedded in the Colifast ALARM and can be downloaded with a universal serial bus (USB) drive. In "at-line" mode, the Colifast ALARM can collect and analyze one sample in approximately 15 hours (h). Colifast provided one unit for testing, limiting the sample capacity to one sample per 24 h. Therefore, the Colifast ALARM was used primarily in manual mode. In manual mode, following the addition of water sample to the sample bottles containing the growth medium, the bottles were incubated in laboratory water baths for the specified timeframes (15-17 h for TC and 14-16 h for EC) before being inserted into the Colifast ALARM for a 30-second fluorescence measurement. While there is no exact time specified at which the samples must be read, the TC samples were removed from the water baths after 15.5 h and the EC samples were removed from the water baths after 14.5 h. It is possible that analysis in manual mode may not be representative of "at-line" mode because of the pre-warming of sample and growth medium and optimal incubation temperature control and during "at-line" analysis within the Colifast ALARM. However, in order for this test to be accomplished in a reasonable time frame, primary use of manual mode was necessary. The results were displayed on the screen in the same way they were for continuous measurements.

VERIFICATION RESULTS

Positive Results. Table 1 summarizes the positive TC test results for the Colifast ALARM.

Table 1. Results Summary for Positive Colifast ALARM Results for TC and EC

TC or EC	Dilution	Colifast ALARM		SM 9221 B/F		Colilert-18	
		+ Results	% of total samples	+ Results	% of total samples	+ Results	% of total samples
TC	B (10 org/100 mL)	7	35%	13	65%	NA	NA
EC	A (50 org/100 mL)	3	15%	9	45%	14	70%

NA-Colilert-18 analyses only performed on EC samples.

Specificity, Sensitivity, FP rate, and FN rate. Table 2 summarizes the specificity, sensitivity, FP rate, and FN rate for TC and EC determined with respect to SM 9221 B and SM 9221 F.

Table 2. Results Summary of Colifast ALARM

Parameter	TC	EC
	Dilution B	Dilution A
Sensitivity	100%	75%
Specificity	100%	100%
False Positive Rate	0%	0%
False Negative Rate	0%	25%

Comparability. A chi-square test for independence with a Yates correction for continuity (because of the small sample size) was performed to compare the Colifast ALARM against the reference methods (SM 9221B for TC, SM 9221F for EC, Colilert-18 for EC). For the Colifast ALARM TC results being compared to the SM 9221B, the chi-square value was less than the critical limit in each case; therefore, for TC, the chi-square test did not detect any differences between the results of the Colifast ALARM and the reference method. In addition, the calculated p-values were also greater than 0.05, indicating that the data did not show a statistically significant difference between the two methods for the detection of TC at the 95% confidence interval and at an 80% power.

For the Colifast ALARM EC results being compared to SM 9221 F, the corrected chi-square value for the EC dilution was less than the critical limit. Therefore, the chi-square test did not detect any differences between the results of the Colifast ALARM and SM 9221F. In addition, the calculated p-values were greater than 0.05, indicating that the data were not significantly different between the two EC methods at the 95% confidence interval. When comparing the Colifast ALARM with Colilert-18, the corrected chi-square value for the EC dilution was more than the critical limit and the calculated p-values were less than 0.05, indicating a significant difference between the two methods at the 95% confidence interval.

Overall, these results were consistent with the power analysis performed before testing and described in Section 5.2 of the report confirming that 20 replicates was adequate to determine significant differences between the methods at 80% power. The determination of smaller differences (1-2 positive results out of 20 replicates) would require additional replicates.

Analysis in "At-line" Mode. The objective of this component of the testing was to verify the Colifast ALARM capability of collecting a sample from a reservoir (which in practice could be almost any container or flowing pipe) and perform the analysis and report results as soon as determined by the Colifast ALARM rather than waiting for the end of the 14 h incubation time period. Duplicate analysis of approximately 30 org/100 mL of EC ATCC 8739 and EC from sewage separated by uncontaminated, filtered water were performed. Both the reference method (SM 9222G-Na-MUG) and the Colifast ALARM did not generate positive EC responses (as evidence by fluorescent colonies) for the ATCC 8739 samples after 4 hr but they did exhibit slight fluorescence after 24 hr, however, the presence of ATCC 8739 organisms in each solution was confirmed with m-Endo plate counts. The sewage samples were determined accurately by the reference method and the Colifast ALARM. When testing the "at-line" mode, adjacent samples with contamination and clean water were analyzed to test the issue of cross-contamination. The sewage EC samples were determined to be positive by the Colifast ALARM after approximately 11 h of incubation.

Operational Factors. The verification staff found that the Colifast ALARM was easy to use. A Colifast ALARM representative came to Battelle to set up the equipment and train the verification staff in the operation of the Colifast ALARM. In manual mode, 100 mL of the water sample were dispensed into each sample bottle containing growth media (separate bottles for TC and EC) and the lids to the bottles were tightened, and the bottles were swirled to dissolve the contents. The cartridges were then placed in a 37-37.5 °C water bath. The vendor instructions called for the TC sample bottles to be incubated for 15-17 h and the EC sample bottles for 14-16 h. During this test, the TC and EC samples were incubated for approximately 15.5 h and 14.5 h, respectively. After the appropriate incubation time, the bottles were removed from the water bath and inserted one at a time into the Colifast ALARM for fluorescence measurement. The bottles were analyzed by clicking on a "start" button on the computer touch screen. The measurement of each sample took approximately 30 seconds.

Incubation of the samples at the correct temperature was critical to obtaining accurate results from the Colifast ALARM. The complete procedure described in the test/QA plan was performed initially with water bath temperatures ranging from 35-36°C. The positive control samples included as part of the ETV test provided negative results suggesting a problem. Upon consultation with Colifast, it was determined that water bath temperatures needed to be in the range of 37-37.5°C. Because of this, the testing was repeated. The results in this report were obtained during the repeated testing and the previous results were not reported since the incubation temperatures utilized were not correct due to a miscommunication with the vendor.

In ”at-line” mode, at least 2.5 L of an EC sample was prepared in order to accommodate for the various rinse cycles that took place for each sample collection. Tubing from the Colifast ALARM was connected to the sample reservoir placed on the bench top next to the Colifast ALARM. The sample analysis was started by clicking on a “start” button on the computer touch screen and the sample was drawn into a sample bottle within the Colifast ALARM with the appropriate growth media being added to the sample bottle. Each sample bottle was incubated for 14 h, and the fluorescence was measured from the sample bottle every hour throughout the incubation to determine if the sample was positive or not. Positive results were immediately indicated by a red light on the outside of the Colifast ALARM, on the screen, and recorded in a text-delimited data file. A positive result could have been reported at any point during the incubation time, while a negative result would not occur until the end of the 14 h incubation. The automated “at-line” mode eliminates the need for a technician to be present to collect analyze and read the water sample result. Also, the Colifast ALARM method calls for a 14 h analysis, shortening the analysis time from the 48 to 72 h required by the standard methods, increasing the efficiency and decreasing the amount of reagents and manpower expended performing the reference methods.

The Colifast ALARM has dimensions of 42 centimeters (cm) wide × 36 cm deep × 64 cm high (17 inches (in) wide × 14 in deep × 26 in high) and weighs approximately 31 kilograms (68 pounds). The Colifast growth media are sold as bottles with 20 tests for the “at-line” mode, or as single sample cartridges. The Colifast ALARM is self contained and does not require any additional equipment or materials to perform analyses. The Colifast ALARM costs approximately $35,000. Sample cartridges can be purchased for approximately $10-15 per sample bottle.

Signed by Tracy Stenner May 16, 2011
Tracy Stenner Date
Manager
Environmental Solutions Product Line
Energy, Environment, and Material Sciences
Battelle

Signed by Sally Gutierrez May 20, 2011
Sally Gutierrez Date
Director
National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency