March 2011

Environmental Technology Verification Report

COLIFAST
ALARM AT-LINE AUTOMATED REMOTE MONITOR

Prepared by

Battelle
The Business of Innovation

Under a cooperative agreement with

EPA **U.S. Environmental Protection Agency**

March 2011

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

COLIFAST
ALARM AT-LINE AUTOMATED REMOTE MONITOR

by

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Notice

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Foreword

The EPA is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

Acknowledgments

The authors wish to thank Jim Sinclair, Sandhya Parshionikar, Mark Rodgers, Jennifer Best, and Keya Sen of the U.S. EPA, and Rick Sakaji of the East Bay Municipal Water District, for their review of the test/QA plan and/or this verification report. Quality assurance (QA) oversight was provided by Michelle Henderson, U.S. EPA, and Rosanna Buhl, Battelle.

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List of Abbreviations

AMS	Advanced Monitoring Systems
ATCC	American Type Culture Collection
ATP	Alternate Test Procedures
BGLB	brilliant green lactose bile
cm	centimeter(s)
COC	chain of custody
DDW	dechlorinated drinking water
DQA	data quality audit
DW	drinking water
EC	*Escherichia coli*
EPA	U.S Environmental Protection Agency
ETV	Environmental Technology Verification
FN	false negative
FP	false positive
h	hour(s)
in	inch(es)
ISO	International Standards Organization
LTB	lauryl tryptose broth
MB	method blank
min	minute(s)
mL	milliliter(s)
MUG	4-methyllumbelliferyl-β-D-glucorinide
N	number of results
n/a	not applicable
NA	nutrient agar
NRMRL	National Risk Management Research Laboratory
org	organism(s)
ppm	parts per million
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
SM	Standard Methods
SSDW	spiked, stressed drinking water
SWTP	Southerly Wastewater Treatment Plant
TQAP	Test Quality Assurance Plan
TC	total coliforms
TCR	Total Coliform Rule
TN	true negative
TP	true positive
TSA	technical systems audit
TSB	trypticase soy broth
USB	Universal Serial Bus

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative 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.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of 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 or 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 EPA's National Risk Management Research Laboratory (NRMRL) and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the ALARM At-Line Automated Remote Monitor by Colifast (Colifast ALARM), a bench top sample collector/analyzer/data logger system for the analysis of total coliforms (TC) and *Escherichia coli* (EC).

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This report provides results for the verification testing of the Colifast ALARM At-Line Automated Remote Monitor (hereafter referred to as the Colifast ALARM). The following is a description of the Colifast ALARM, based on information provided by the vendor.

Figure 2-1. Colifast ALARM

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 or EC. The Colifast ALARM method is based on an enzymatic reaction. The Colifast TC medium contains the substrate 4-metylumbelliferyl (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-metylumbelliferyl (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 reactions. The media contains inhibitors to hinder growth of non-coliforms.

A 100 mL water sample is added to a sample bottle,incubated, 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. The Colifast ALARM is shown in Figure 2-1 and the instrument software displayed during a run in the embedded Colifast ALARM touch screen computer is shown in Figure 2-2.

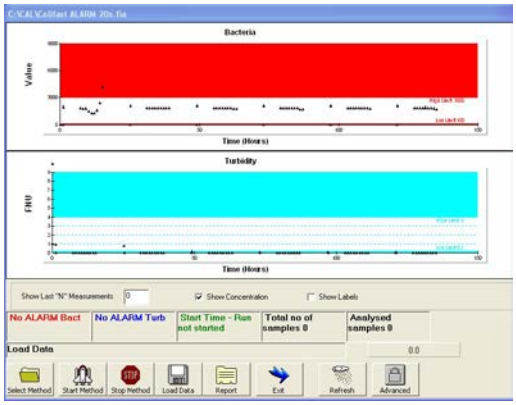

Figure 2-2. Colifast ALARM Display

When the Colifast ALARM is operated in "at-line" mode, 100 mL samples are collected automatically using the pump system. The sample can be collected from any source (flowing or static) reachable by the flow injection pump system. The analysis is then peformed autmatically and the software within the Colifast ALARM automatically interprets the fluorescent measurements hourly throughout the incubation time and a positive result is reported on the screen and by audio/ visual alarms when the presence of TC or EC is detected, regardless of the amount of time that has passed. In addition, Colifast has the capability to provide results by industrial interface (relays) and mobile networks. The results are stored on the computer provided with the Colifast ALARM and can be downloaded with a universal serial bus (USB) drive or accessed via local area network remote control. In "at-line" mode, the Colifast ALARM can collect and analyze TC samples in approximately 15 hours (h) and EC samples in 14 h, and have a total capacity of 20 analyses per run.

The "at-line" operation mode for the Colifast ALARM allows for one sample every 16 hours, but for the convenience of the staff that was spiking the samples it was programmed to do one sample analysis every 24 hours. Colifast provided one unit for testing which limited the sample capacity to one sample per 24 h. The large number of samples required for this verification test exceeded that capacity. 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 fluorescent 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 entirely representative of the "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 timeframe primary use of manual mode was necessary. The results were displayed on the screen in the same way they were for the continuous measurements.

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 ALARM has dust and water resistant enclosure and is equipped with transport handles to facilitate moving around and installation at various locations.
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.

Chapter 3
Test Design and Procedures

3.1 Introduction

The ETV AMS Center Water Stakeholder Committee identified the use of coliform detection technologies for the monitoring of drinking water (DW) as an area of interest for technology verification. Fecal pollution can introduce disease-causing (pathogenic) bacteria, viruses, and parasites into receiving waters, which may serve as private/public DW supplies. Utilities fully recognize the possibility of this waterborne pollution and take every precaution (filtering, treatment with disinfectants such as chlorine and chloramines, and regulatory compliance sampling and analysis) to avoid fecal contamination in DW. Based on the U.S. EPA's 1989 Total Coliform Rule (TCR)[1], assessment of this health risk is based on the detection and enumeration of fecal indicator bacteria, such as TC and EC, whose presence indicates a potential pathway for contamination (e.g., sewage or animal waste) of the distribution system which is designed to provide a physical barrier to contamination of DW. It is important to note that this verification test was not being conducted to provide data to be used to approve technologies for use in meeting regulatory requirements for the detection of TC or EC as required by either the 1989 TCR or the anticipated revision to the TCR. It was conducted, based on feedback from ETV AMS Center stakeholders, to provide a verification test that is similar in requirements to the current TCR approval protocol (referred to as the Alternative Testing Procedures (ATP) protocol)[2], such that technologies that are not already approved have an opportunity to be tested under a similar set of test conditions.

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. The criteria for ETV cooperation are outlined in a cooperative verification process document prepared by the respective cooperating ETV programs. It should be noted however that neither U.S. ETV verification, nor the cooperation with the ETV Canada or DANETV programs, represents an approval of methods for regulatory compliance.

3.2 Test Overview

This verification test was conducted according to procedures specified in the Test/QA Plan for Verification of Coliform Detection Technologies for Drinking Water[3] (TQAP) and adhered to the quality system defined in the ETV AMS Center Quality Management Plan (QMP)[4]. As indicated in the test/QA plan, the testing conducted satisfied EPA QA Category II requirements. The test/QA plan and/or this verification report were reviewed by:

- Rick Sakaji, East Bay Municipal Water District
- John Neate, Strategies for Change

- Mona El Hallak, OCETA
- Claus Jørgensen, DHI Group
- Jim Sinclair, U.S. EPA
- Sandhya Parshionikar, U.S. EPA
- Jennifer Best, U.S. EPA
- Keya Sen, U.S. EPA
- Mark Rodgers, U.S. EPA (test/QA plan only).

The TCR sets both goals and legal limits for the presence of TC and EC in DW. To summarize, the TCR states that the objective is for zero TC organisms in DW samples and the rule (for large water systems) is that no more than 5% of all DW samples collected by a utility can be positive. 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). While it is difficult to determine if a single target organism is present in 100 mL of water, the ATP protocol suggests that 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 test, the objective was to use the ATP protocol as a guide to prepare spiked DW dilution sets that provided 50 ±25% positive results for both TC and EC with the reference method(s) and then compare the results from the reference method to those from the tested technology.

Similar to the TCR in Europe, the Official Journal of the European Communities published Council Directive 98/83/EC in 1998 that provided directives on the regulation of public water systems within the European Union. Annex IA within that document includes the requirement for zero EC per 100 mL. The European Drinking Water Directive prescribes minimum sampling and analysis frequencies with the use of ISO 9308-1[5], the ISO procedure for measurement of coliforms, or an equivalent method for compliance monitoring of EC. In order to be accepted as equivalent, the alternative method must be compared to ISO 9308-1 according to ISO 17994[6], the ISO procedure for showing method equivalence. ISO 9308-1 provides both a measurement of the presence or absence of E-coli or total coliform bacteria, and number of bacteria. Colifast ALARM only provides a test of presence or absence of EC and TC. Recently, the Colilert-18 combined with QuantiTray for quantification has been shown to be equivalent[7] with ISO 9308-1. 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.

In this report, results from the Colifast ALARM were compared to the results obtained from the reference method analyses which were presence/absence methods for TC and EC, specifically, Standard Methods (SM)[8] 9221B (TC) and 9221F (EC). In addition, the EC results were also compared to the Colilert-18 presence/absence method. The SM and Colilert 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 8, 2010 at Battelle in Columbus, Ohio with the reference method analyses being performed at Superior Laboratories in Galloway, Ohio (which is a 20 minute drive from Battelle). 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 which includes the comparison of false positive rate and false negative rate. In addition, sustainable operational factors such as ease of use, required reagents, analysis time, and laboratory space and utilities required are reported.

3.3 Experimental Design

3.3.1 Verification Test Sample Preparation

The preparation of verification test samples included the collection of raw sewage as the source of the target organisms, collection of the DW sample, the fortification of the DW sample with target organisms, and the chlorine stressing and dilution of samples for analysis. A detailed description of the sample preparation steps is provided in the TQAP. A summary of the sample preparation activities and timeline is provided below.

3.3.1.1 Sewage and Drinking Water Sample Collection

A single raw sewage sample (approximately 0.6 liter (L)), was collected at 7 A.M. August 31, 2010 at the Southerly Wastewater Treatment Plant (SWTP) in Columbus, Ohio. The sewage sample was a 24 h composite sample collected automatically over a 24 h period (midnight August 30 – midnight August 31). The SWTP automated system collects 50-100 mL aliquots at approximately 5 minutes intervals directly into a refrigerated carboy. The sewage sample was collected from this carboy. The sampling approach was a deviation from the TQAP, which had implied that the sample would be collected without compositing. Battelle believes that there was not an adverse impact to the results of the evaluation due to this deviation because the coliform levels were adequate for the purposes of testing.

Upon sampling, the sewage sample was immediately stored on wet ice, and transported by Battelle staff to Battelle laboratories. Upon receipt, the sewage sample was filtered through a Whatman No. 2 filter (11 micron pore-size) under vacuum using a Buchner funnel to remove excess solids, shaken vigorously for 1 minute to insure homogeneity, and then immediately characterized for total culturable heterotrophic bacteria, TCs, and EC.

A single DW sample was collected from the tap at the Battelle laboratory the same day the sewage sample was collected. The DW sample was collected by first removing the faucet screen and decontaminating the surface with 70% isopropanol. Next, the line was purged for 3 minutes with cold water and 80 L of DW were collected from the tap into multiple sterile (autoclaved) carboys equipped with a spigot and containing large stir bars. Once collected, aliquots from each carboy were pooled and then used to characterize the DW using the methods and standard operating procedures provided in Table 3-1. Table 3-1 also gives the results of the initial characterization of the sewage and DW samples.

Table 3-1. Methods, Equipment, and Results for the Characterization of Sewage and Drinking Water Samples

Parameter	Units	Equipment/Media	SOP/Method	Sewage	DW
pH	n/a	calibrated pH meter	SOP GEN.V-003-10[9]	n/a	7.7
temperature	°C	calibrated thermometer	SOP GEN.V-013-047[10]	n/a	24
free chlorine	mg/L	HACH Chlorine test kit	HACH Method 8021	n/a	1.2
total chlorine	mg/L	HACH Chlorine test kit	HACH Method 8167	n/a	1.2
total, culturable heterotrophic bacteria	org/100 mL	R2A agar	AOAC's Bacteriological Analytical Manual[11]	5.6×10^6	n/a
TC	org/100 mL	m-Endo	SM 9222B	1.6×10^7	n/a
EC	org/100 mL	NA-MUG	SM 9222G	2.7×10^6	n/a

n/a - not measured
NA - Nutrient agar
MUG 4-methyllumbelliferyl-β-D-glucorinide

3.3.1.2 Chlorine Stressing and Preparation of Samples for Verification Testing

The Colifast ALARM was tested with chlorine stressed TC and EC. The chlorination stressing step was started within 3 h from the time Battelle received the sample, or approximately 10 h from the time the last automated sample was collected and 34 h from the time the first automated sample was collected. This multi-step stressing process was accomplished on the same day as DW sample collection by adding approximately 40 L of the unspiked DW sample to one 50 L carboy. The DW was adjusted to a free chlorine concentration of 2 parts per million (ppm) using a 4% hypochlorite solution, after which 10.5 L was dispensed into three 10L aliquots containing stir bars. Each aliquot was then spiked with TC and EC by adding 200 mL of filtered sewage (amount of sewage providing enough TC and EC to bring the DW sample to a starting concentration of approximately 10^5 TC org/100 mL and 10^4 EC org/100 mL). Based on pre-testing range finding experiments, the three aliquots were chlorinated for 20, 40, and 60 seconds, respectively, after which time the samples were dechlorinated with sodium thiosulfate and subsequently enumerated using SM9222 B and G. The results determined the log reduction of TC and EC due to the chlorine stressing that had occurred in each aliquot. This chlorine stressing step was considered adequate if the number of organisms in the spiked DW samples was reduced by two to four orders of magnitude.

During the testing of the Colifast ALARM, the 20 second chlorine stressing attained a two log reduction in both TC and EC so after having been refrigerated overnight, that aliquot of spiked, stressed drinking water (SSDW) was used to prepare the diluted samples for analysis. To test the coliform technologies, separate SSDW samples of TC and EC containing concentrations of approximately 1 org/100 mL needed to be prepared. Based on preliminary work with similar sewage samples, EC concentrations were approximately 10 times less than the TC concentrations. To ensure that concentrations of approximately 1 org/100 mL would be attained for both TC and EC, a range of concentrations were prepared. Three separate aliquots, approximately 10 L each, of dechlorinated DW (DDW) were added to carboys and spiked with a calculated volume of SSDW sample to generate target suspensions of 5 TC/100 mL, 10 TC/100 mL, and 50 TC/100 mL. Each dilution was mixed on a stir plate for 5 to 10 minutes, and then, as

mixing continued, 100 mL aliquots were dispensed into sterile 100 mL bottles using 50 mL and/or 100 mL graduated pipettes. Twenty replicate samples were prepared at each concentration level. Once all 100 mL aliquots were dispensed for technology verification (20 at each dilution level for a total of 60 replicates), verification testing was initiated. All samples were stored refrigerated during the day of preparation until the analysis was initiated that same day. These concentration levels were changed through a deviation in the TQAP because these concentration levels offered an increased likelihood that the targeted ratio of positive and negative results would be obtained from the reference method.

In addition to the samples to be used for Colifast ALARM verification, a set of twenty 100 mL aliquots were prepared for the reference method analysis. Immediately after being dispensed, all reference samples were transported by car in coolers packed with ice packs to Superior Laboratories, Inc. Sample custody for all samples transferred to Superior Laboratories were documented using a chain-of-custody (COC) form following Battelle SOP ENV-ADM-009 for Chain of Custody[12]. The COC form was signed once receipt of all samples had been confirmed. Reference method analysis was initiated on the same day as arrival at the laboratory, within 2 h of initiation of the Colifast ALARM sample analysis.

3.3.2 Sample Analysis

The ability of the Colifast ALARM to determine the presence of TC and EC was challenged using 20 replicates of the three concentrations of SSDW samples. The number of replicates was determined after performing a power analysis with a fixed 80% power (described more thoroughly in Section 5.2). Positive/negative control samples spiked with quality control (QC) cultures listed in Table 3-2 as well as method blank samples were included during testing. One Colifast ALARM was provided to perform all of the replicate samples shown in Table 3-3. Because of the large number of concurrent samples analyses required during this verification test, the samples were incubated apart from the Colifast ALARM and then analyzed in the Colifast ALARM one at a time after incubation periods (15.5 h TC and 14.5 h EC) for fluorescent measurement. All of the samples were assayed by the reference methods and the Colifast ALARM concurrently.

Table 3-2. Quality Control Strains

Targeted Coliform	Method Blank	Positive Control	Negative Control
TC	Sterilized DW	*Enterobacter aerogenes* ATCC 13048 *Escherichia coli* ATCC 8739	*Pseudomonas aeruginosa* ATCC 10145
EC	Sterilized DW	*Escherichia coli* ATCC 8739	*Enterobacter aerogenes* ATCC 13048 *Pseudomonas aeruginosa* ATCC 10145

ATCC - American Type Culture Collection

Table 3-3. Replicate Samples by each Analysis Method

Sample Description	Replicate Analyses by Colifast ALARM	Replicate Analyses by SM9221B	Replicate Analyses by SM9221F	Replicate Analyses by Colilert-18
Dilution A – approx. 50 TC/100 mL	20	20	20	20
Dilution B – approx. 10 TC/100 mL	20	20	20	20
Dilution C – approx. 5 TC/100 mL	20	20	20	20
Method Blank	3	3	3	3
TC Positive control	3	3	3	3
EC Positive control	3	3	3	3
Negative control	3	3	3	3
Total Replicate Analyses	72	72	72	72

3.3.2.1 Confirmation of Results

The SM 9221B and 9221F reference methods and Colifast ALARM results were confirmed with more definitive tests in order to adequately verify the Colifast ALARM. Confirmation for the SM 9221B and 9221F reference methods, as well as the Colifast ALARM, is described in detail in the TQAP. In summary, for the Colifast ALARM analyses, 1 mL of each 100 mL sample resulting from the 15.5 h (for TC) and 14.5 h (for EC) Colifast ALARM incubation was inoculated into 9 mL of lauryl tryptose broth (LTB) and analyzed using SM 9221B and 9221F. Following the LTB step, TCs were confirmed using brilliant green lactose bile (BGLB) broth, and EC were confirmed using EC-MUG. Figure 3-1 illustrates the process by which all positive and negative samples from the Colifast ALARM and SM 9221B and 9221F were confirmed. As an additional, optional confirmation, a complete test for TC was performed for several samples by inoculating MacConkey media and then selecting suspected TC colonies and inoculating into LTB, as described by SM 9221B.

3.3.3 Analysis in "At-line" Mode

An optional component of the ETV test was performed to verify the capability of the Colifast ALARM to detect EC ATCC 8739 in "at-line" mode which provides positive results as soon as determined by the Colifast ALARM. The "at-line" operation mode for the Colifast ALARM allows for one sample every 16 hours, but for the convenience of the staff that was spiking the samples it was programmed to do one sample analysis every 24 hours. Therefore, only four analyses containing EC were performed. Two analyses of EC ATCC 8739 at a concentration of approximately 30 EC per 100 mL each followed by 1-3 filter sterilized water samples. More than one filter sterilized water sample was analyzed when these experiments were set to run over the weekend. Following the initial intake of an EC sample on Friday, the filter-sterilized water sample was connected to the Colifast ALARM and was analyzed repeatedly until the sample was switched at the start of the following week. This procedure was repeated with EC at a similar concentration level, but using EC from sewage water rather than from a pure culture. This was a deviation from the TQAP because the details of the approach to this testing for the Colifast ALARM had been inadvertently omitted. The EC ATCC 8739 samples were also analyzed by using a quantitative method for EC (SM 9222G – Na-MUG).

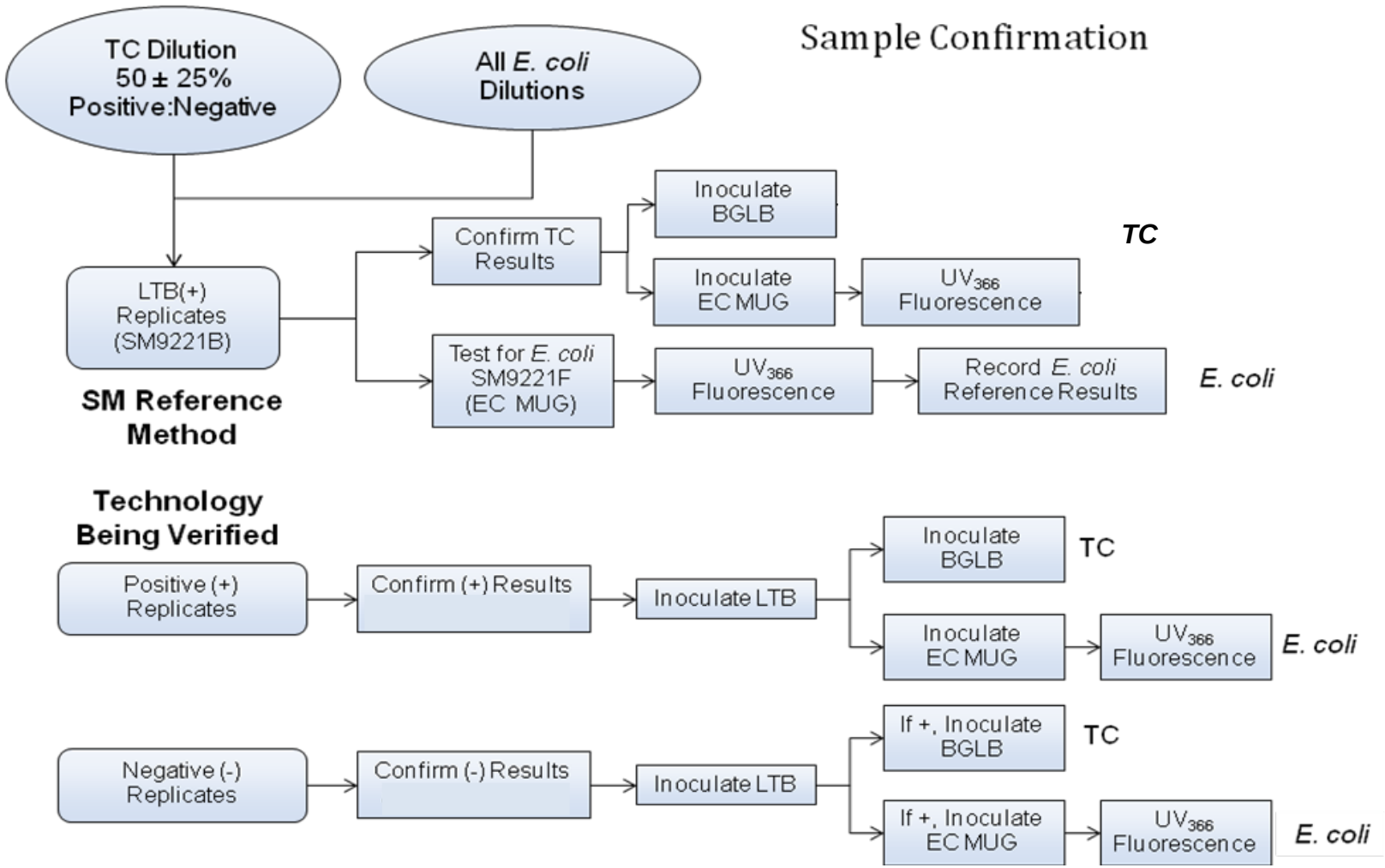

Figure 3-1. Flowchart describing confirmation analyses for both the Colifast ALARM and SM9221B and F

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the TQAP for this verification test[1] and the QMP for the AMS Center[2]. QA/QC procedures and results are described in the following subchapters.

During testing, there were three deviations from the TQAP. The first was described in Section 3.3.1.1 and involved a change in collection method for the sewage sample. The TQAP had implied that the sewage sample would be sampled directly and not composited over two days. The second deviation was described in Section 3.3.1.2 and changed the concentrations of the test samples in order to provide a better likelihood that the target ratio of positive and negative results be obtained from the reference method. The third deviation was described in Section 3.3.3 and clarified the approach to the additional optional testing of the "at-line" feature of the Colifast ALARM as it had been previously omitted. These deviations were judged by the Battelle Verification Test Coordinator to not result in any adverse impacts on the quality of the data generated. The deviations were reviewed and approved by the EPA ETV AMS Center Project Officer and EPA ETV AMS Center Quality Manager.

4.1 Quality Control Samples

The reference method required the use of method blanks (MB), positive and negative control organisms, and result confirmation. One MB was performed during the analysis for every 20 samples analyzed. The MB consisted of 100-mL dechlorinated, sterilized tap water processed as a sample. MB samples were exposed to identical handling and analysis procedures as the rest of the test samples, including the addition of all reagents. These samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. All three MB samples analyzed by the Colifast ALARM as well as the reference methods were negative, indicating the absence of TC and EC.

Three positive and negative control samples were also be analyzed using the Colilert-18 and SM 9221B/F reference methods. Positive and negative ATCC control cultures were purchased from MicroBioLogics. Control organisms included the TC *Enterobacter aerogenes* (ATCC 13048), EC (ATCC 8739), and the non-coliform *Pseudomonas aeruginosa* (ATCC 10145). All control cultures were prepared onto tryptic soy agar and incubated overnight. The QC control samples were then prepared by inoculating triplicate 100 mL filter sterilized DDW aliquots each with 1 mL of a slightly turbid culture suspension prepared from the agar cultures in DDW. Control samples were used to confirm the accurate response (positive response for positive control and negative response for the negative controls) of the Colifast ALARM and reference methods at relatively high concentrations. The control cultures were approximately 10^6 org/100 mL.

All three TC positive controls were determined to be positive using the reference methods and the Colifast ALARM (and confirmed to be positive during the applicable confirmation analyses). In addition, all three EC positive controls were determined to be positive (for both TC and EC) using the reference methods and the Colifast ALARM (and confirmed to be positive during the confirmation analysis). All three TC negative control samples were found to be negative for TC during the Colifast ALARM and reference analyses. All three EC negative control samples were found to be negative for EC during the Colifast ALARM and reference analyses.

4.2 Audits

Two types of audits were performed during the verification test; a technical systems audit (TSA) of the verification test procedures, and a data quality audit (DQA). Audit procedures for the TSA and the data quality audit are described further below.

4.2.1 Technical Systems Audit

The Battelle AMS Center Quality Manager performed a TSA on July 20, 21, and 22, 2010 at Battelle's microbiology laboratory in Columbus, OH and at the reference laboratory, Superior Laboratories in Galloway, OH during the initial verification tests. The EPA AMS Center Quality Manager participated in the Battelle and Superior Laboratories audits on July 21. The TSA consisted of interviews with Battelle and Superior Laboratories personnel, observations of test sample preparation and testing at Battelle and Superior Laboratories, and observation of sample analysis. The purpose of the audit was to verify that:

- Sample preparation procedures were performed by Battelle according to the TQAP requirements
- Reference laboratory methods for analyzing test samples conformed to the TQAP and reference method requirements
- Technology testing was performed according to the TQAP and vendor instructions
- Test documentation provided a complete and traceable record of sample preparation and analysis
- Equipment used in the test was calibrated and monitored according to TQAP requirements and standard laboratory procedures.

Seven (7) Findings, six (6) Observations, and three (3) Remarks were identified during the TSA. The findings involved training records, reference method requirements, sewage sample collection, sample custody, and traceability of critical reagents. It was determined by Battelle that none of these had an adverse impact on the test results and all findings have received a satisfactory response.

In response to this audit report, the following actions were taken:

- Documentation of reference laboratory microbiology training was provided;
- Generation of a deviation to more accurately describe the collection of the sewage water sample;
- Clarified and added detail to the documentation of sewage sample collection on the custody form.

A TSA report was prepared and distributed to EPA.

4.2.2 Data Quality Audit

Records generated in the verification test received a one-over-one review before these records were used to calculate, evaluate, or report verification results. Data were reviewed by a Battelle technical staff member involved in the verification test. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

In addition, audits of data quality (ADQs) were conducted on October 6 and 7, 2010. During the audits, laboratory data generated at the reference laboratory, Superior Laboratories, Inc. and data generated by the Colifast ALARM were reviewed and verified for completeness, accuracy and traceability. The verification of coliform detection technologies was determined by the EPA AMS Center Project Officer to be Category II test. Accordingly, at least 25% of the results for each of the testing scenarios were verified versus the raw data, and 100% of the QC sample results were verified. The data were traced from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

Three (3) Findings and three (3) Observations were identified during the ADQs. The three findings involved sample custody, missing test data, and changes in the design of the optional "at-line" analysis. Battelle believes that none of these had an adverse impact on the test results and all have received a satisfactory response.

In response to these audit reports, the following actions are anticipated:

- Custody forms amended to accurately reflect sample transfers;
- Laboratory documentation provided to verify missing data point;
- "At-line" procedure clarified.

A data audit report was prepared and distributed to EPA.

Chapter 5
Statistical Methods

The statistical methods used to evaluate the quantitative performance factors are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

5.1 False Positive Rates, False Negative Rates, Sensitivity, and Specificity

False positive (FP) and false negative (FN) rates of the Colifast ALARM were evaluated when assessing comparability. During this test, true positives (TP) were those positive results from the Colifast ALARM that were confirmed, and false positives were those positive results from the Colifast ALARM that were not confirmed by the reference method. Conversely, true negative (TN) results were those negative results that were confirmed as negative, and false negative results were those negative results that were shown to be positive by the confirmatory method. Performance of the Colifast ALARM was tested by comparing the proportion of true positive results from those technologies to the proportion of positive results from the SM 9221B and F reference methods.

Sensitivity is defined as the percent of positive samples correctly identified as positive and specificity is defined as the percent of negative samples correctly identified as negative. Estimates of sensitivity, specificity, false positive rates, and false negative rates as percentages for the two methods were calculated as follows:

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100\%$$

$$\text{False positive rate} = \frac{FP}{TN+FP} \times 100\% = \left(1 - \frac{TN}{TN+FP}\right) \times 100\% = 1 - \text{Specificity}$$

$$\text{False negative rate} = \frac{FN}{TP+FN} \times 100\% = \left(1 - \frac{TN}{TN+FP}\right) \times 100\% = 1 - \text{Sensitivity}$$

5.2 Method Comparability

In order to assess whether the proportion of positive and negative samples were significantly different between the Colifast ALARM and the reference method, chi-square tests for independence were conducted. The chi-squared test was modeled in SAS® (ver. 9.1.3), using the FREQ procedure. Because of the small sample size (some dilutions had less than five

positive or negative results); the Yates continuity correction was applied. If the corrected chi-square value was less than the critical value, the sample results between the two methods were not considered significantly different (95% confidence, alpha = 0.05, p-value > 0.05). If the corrected Chi-square value is greater than the critical value (p-value $\leq$ 0.05), the results between the two methods were considered to be significantly different. It should be noted that the Yates continuity correction is a more conservative statistical approach, making it less likely that a significant difference would be determined when it does not exist.

Prior to testing, a power analysis was conducted to determine the number of replicates required to determine possible significant differences between the technologies being tested and the reference method. The power analysis was done assuming that the total number of tests, while not limited, would be the same for both the technology being tested and the reference method and that the standard deviations of each would be equal. Conducted using the POWER procedure in the SAS System, the power analysis determined the number of replicate tests (across both test types) that would be necessary to detect a specified difference in proportions of a specified size with fixed 80% power, given a specified value of the proportion for the reference test (the acceptable range of reference test positive proportions was 25% to 75% for this test), and a significance level of 0.05 for the test. To summarize, the power analysis shows that for approximately 20 replicates, if the reference method was 25% positive (5 positive results and 15 negative results), then the technology being tested would be required to be 65% positive (13 positives and 7 negative results) to have a significant difference. Colifast ALARM results with a lesser percentage of positive results out of 20 replicates would be considered similar to the reference method. Similarly, if the reference method was 50% positive, then a significant difference could be determined with Colifast ALARM results that were less than 2 positives and 18 negatives or more than 18 positives and 2 negatives. Finally if the reference method was 65% positive, then a significant difference could be determined with at most a 32% positive result (6 positives and 14 negatives). The Colifast ALARM results are discussed in the context of this power analysis.

In summary, the smallest difference that is able to be determined with 20 replicates is a relative difference of 6-8 positive results. The power analysis revealed that differences of 1 or 2 positive results could be determined, but between 150 and 1,250 replicates may be required.

Chapter 6
Test Results

As mentioned previously, this verification test included both quantitative and qualitative evaluations. The quantitative evaluation was conducted to assess the comparability of results generated by the presence/absence results for the Colifast ALARM with those generated by the presence/absence result from the reference methods. The qualitative evaluation was performed to document the operational aspects of the Colifast ALARM when it was used during verification testing. The following sections provide the results of the quantitative and qualitative evaluations. Tables presenting the raw data presence/absence results for the reference methods, the Colifast ALARM, and the confirmation analyses are provided in the Appendix.

6.1 TC Data

The positive TC test results for the Colifast ALARM and reference method (SM 9221B) are presented in Table 6-1. One of the three dilutions (Dilution B) yielded the target 50 ± 25% split in responses for SM9221B.

Table 6-1. TC Positive Results

Dilution (target TC conc.)	Colifast ALARM		SM 9221B	
	+ Results	% of total samples	+ Results	% of total samples
B (10 org/100 mL)	7	35%	13	65%

N – Number of results

Tables 6-2 and 6-3 summarize the TP (confirmed) and TN (confirmed) TC results for the Colifast ALARM. The reference method data are also presented.

Table 6-2. TC Data Summary - Positives

Dilution (target TC conc.)	Colifast ALARM			SM 9221B
	+ Results	Confirmed TP	Difference (FP)	+ Results
B (10 org/100 mL)	7	7	0	13

Table 6-3. TC Data Summary - Negatives

Dilution (target TC conc.)	Colifast ALARM			SM 9221B
	- Results	Confirmed TN	Difference (FN)	- Results
B (10 org/100 mL)	13	13	0	7

The sensitivity, specificity, false-positive, and false-negative rates for the Colifast ALARM TC with respect to SM 9221B were determined as described in Section 5.1 and are presented in Table 6-4.

Table 6-4. TC Data Summary – Confirmations [a]

Parameter	Dilution B
Sensitivity	100%
Specificity	100%
False Positive	0%
False Negative	0%

[a]Results calculated with respect to SM 9221B as the reference method (see Section 5.1).

6.2 EC Data

Table 6-5 summarizes the positive EC test results for the Colifast ALARM analyzed according to the manufacturer's directions. The positive EC test results for the reference methods (SM 9221F and Colilert-18) are also presented. One of the three dilutions (Dilution A) yielded 45% positive results for SM9221F, which was within the target range of 50 ± 25%, so only results from Dilution A are reported.

Table 6-5. EC Positives

Dilution (target TC conc.)	Colifast ALARM		SM 9221F		Colilert-18	
	+ Results	% of total samples	+ Results	% of total samples	+ Results	% of total samples
A (50 org/100 mL)	3	15%	9	45%	14	70%

Tables 6-6 and 6-7 summarize the confirmed TP and TN EC results for the Colifast ALARM. The reference method data are also presented.

Table 6-6. EC Summary – Positives

Dilution (target TC conc.)	Colifast ALARM			SM 9221F	Colilert-18
	+ Results	Confirmed TP	Difference (FP)	+ Results	+ Results
A (50 org/100 mL)	3	3	0	9	14

Table 6-7. EC Summary – Negatives

Dilution (target TC conc.)	Colifast ALARM			SM 9221F	Colilert-18
	- Results	Confirmed TN	Difference (FN)	- Results	- Results
A (50 org/100 mL)	17	16	1	11	6

The sensitivity, specificity, false-positive, and false-negative rates for the Colifast ALARM EC results with respect to SM 9221F were determined as described in Section 5.1 and are presented in Table 6-8.

Table 6-8. EC Data Summary – Confirmations [a]

Parameter	Colifast ALARM
Sensitivity	75%
Specificity	100%
False Positive	0%
False Negative	25%

[a] Results calculated with respect to SM 9221F as the reference method (see Section 5.1)

6.3 Method Comparability

Table 6-9 shows the results from the chi-square test for independence that was performed to compare the TC results from the Colifast ALARM for each incubation time period against the reference method SM 9221B. Because of the small number of replicates the Yates continuity correction was performed on the chi-square results. When comparing the Colifast ALARM results to the SM 9221B reference method, the corrected chi-square value for the TC 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 9221B. The p-value was greater than 0.05, indicating that the data did not show a statistically significant difference between the two methods for the detection of TCs at the 95% confidence level.

Table 6-9. TC – SM 9221B

Dilution (target TC conc.)	Colifast ALARM		SM9221B		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
B (10 org/100 mL)	7	13	13	7	2.500	1	0.114	3.841

These results are consistent with the power analysis performed before testing and described in Section 5.2. For TC, the SM 9221B reference method generated an observed result of 13 positive results out of 20 for Dilution B. According to the power analysis, when 22 replicates are included in the experiment (we included 20), an observed result of 6 or less positive results out of 20 from the Colifast ALARM would be required for the result to indicate a significant underlying difference between the reference method and the Colifast ALARM. Therefore, the observed 7 positive results out of 20 from the Colifast ALARM indicated there was not a significant underlying difference indicated.

Tables 6-10 and 6-11 show the results from the Yates corrected chi-square test for independence that was performed to compare the EC results from the Colifast ALARM against both reference methods (SM 9221F and Colilert-18). When comparing the Colifast ALARM results to the SM 9221F reference method, the chi-square value was also less than the critical limits; therefore, the chi-square test did not detect any differences between the results of the Colifast ALARM and the reference method SM 9221F. 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 detection of EC.

When comparing to the Colilert-18 reference method, the corrected chi-square value was greater than the critical limits; therefore, the chi-square test determined a significant difference between the results of the Colifast ALARM and Colilert-18. In addition, calculated p-values were less than 0.05, indicating a statistically significant difference between the two methods for detection of EC.

Table 6-10. EC – SM 9221F

Dilution (target TC conc.)	Colifast ALARM		SM9221F		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (50 org/100 mL)	3	17	9	11	2.976	1.000	0.0845	3.841

Table 6-11. EC – Colilert-18

Dilution (target TC conc.)	Colifast ALARM		Colilert-18		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (50 org/100 mL)	3	17	14	6	10.23	1.000	0.0014	3.841

As was the case for TC, the EC results are consistent with the power analysis performed before testing. The proportion of observed positive results from the SM 9221F reference method was 45% (9 positive and 11 negative). According to the power analysis, an observed result of approximately 5% positive result (1 positive or less out of 20 results) would be required from the Colifast ALARM for a significant underlying difference to be determined between the reference method and the Colifast ALARM. The observed result from the Colifast ALARM was 3 positive results out of 20 so there was not a significant underlying difference determined.

In comparing the EC result from the Colifast ALARM to the Colilert-18 reference method, the observed results from Dilution A resulted in 70% positive results from the Colilert-18 reference method. According to the power analysis, when the reference method has an observed rate of 75% positive (15 positive and 5 negative), the observed results from the Colifast ALARM would require 13 or more negative results out of 20 to indicate a significant underlying difference from the reference method. The Dilution A Colifast ALARM observed result was 85% negative (3 positive and 17 negative) so a significant underlying difference was indicated.

6.4 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 an incubation time period. Table 6-12 gives the results for the

Table 6-12. Results of Analysis in "At-line" Mode

Sample Description	m-Endo plate counts (EC/100mL)	SM 9222G-NA-MUG (EC/100mL)	EC X=Presence O=Absence	Incubation Time/EC Detected
EC ATCC 8739	38	0	O	14 h
FS-DDW	n/a	n/a	O	14 h
FS-DDW	n/a	n/a	O	14 h
EC ATCC 8739	33	0	O	14 h
FS-DDW	n/a	n/a	O	14 h
EC from sewage	36	36	X	11 h
FS-DDW	n/a	n/a	O	14 h
FS-DDW	n/a	n/a	O	14 h
FS-DDW	n/a	n/a	O	14 h
EC from sewage	28	28	X	11 h
FS-DDW	n/a	n/a	O	14 h

X=Presence; O= Absence; n/a = not analyzed
FS-DDW – filter sterilized dechlorinated drinking water

analysis of approximately 30 org/100mL of EC ATCC 8739 and EC from sewage separated by uncontaminated filter sterilized water. 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. All of the FS-DDW water samples were negative for EC. The sewage EC samples were determined by the Colifast ALARM to be positive after approximately 11 hours of incubation.

6.5 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. The Colifast ALARM was set up by plugging the Colifast ALARM and powering up. For operation in continuous mode, no special laboratory facilities were required. In manual mode, laboratory water baths were required. Following an approximately 30 minute training session, the operators (consisting of Battelle microbiology technicians) were comfortable operating the Colifast ALARM without assistance.

As previously described, the Colifast ALARM was operated in manual measurement mode for the measurement of TC and EC. 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 lid to the bottle was tightened and then swirled to dissolve the contents. The cartridges were then placed in a water bath that was held between 37-37.5 °C. The vendor instructions call for the TC sample bottles to be incubated for 15-17 hours and the EC sample bottles for 14-16 hours. During this test, the TC samples were incubated for approximately 15.5 hours and 14.5 hours, 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 fluorescent measurement. The bottles were analyzed by clicking on a "start" button on the computer 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 (which was demonstrated for EC only because of limited time), at least 2.5 L of 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 with a tap on the start button on the touch screen computer and the sample was drawn into a sample bottle within the Colifast ALARM and the appropriate growth media was added to the sample bottle. This sample bottle was incubated for 14 hours. Every hour throughout that incubation, the fluorescence was measured from the sample bottle to determine if the sample was positive or not. Positive results were indicated by a red light on the outside of the Colifast ALARM, by audio 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 the water sample, analyze and read the sample result. Also, the Colifast ALARM method calls for a 14 h analysis, shortening the analysis time from the 48 to 72 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.

Chapter 7
Performance Summary

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 per 100 mL samples. This ETV test verified the performance of the Colifast ALARM at that level of detection. 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 near single organism detection. Therefore, for the purpose of this verification test, spiked DW dilution sets were prepared that provided 50 ±25% positive results for TC and EC with the reference methods and then the results from the reference methods were compared with the Colifast ALARM. The results of the verification of the Colifast ALARM are summarized below:

Positive Results. Table 7-1 summarizes the positive TC test results for the Colifast ALARM.

Table 7-1. Results Summary for Positive Colifast ALARM Results for TC and EC

TC or EC	Dilution	Colifast ALARM		SM 9221B/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 (50org/100 mL)	3	15%	9	45%	14	70%

NA – Colilert-18 analyses were only applicable to the EC samples

Specificity, Sensitivity, FP rate, and FN rate. Table 7-2 summarizes the specificity, sensitivity, FP rate, and FN rate for TC and EC with respect to SM 9221B and 9221F. Sensitivity is defined as the percent of positive samples correctly identified as positive and specificity is defined as the percent of negative samples correctly identified as negative.

Table 7-2. 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%

Results calculated with respect to SM 9221B and F as the reference methods for TC and EC, respectively.

Comparability. In addition, 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, 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, the chi-square test did not detect any differences between the results of the Colifast ALARM and the reference method for TC. 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.

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 for EC. 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 EC at the 95% confidence interval. When comparing 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 that the data did show a statistically significant difference between the two methods for the detection of EC at the 95% confidence interval.

Overall, these results were consistent with the power analysis performed before testing and described in Section 5.2 in that the results confirmed that 20 replicates was adequate to determine significant differences (differences of 6-8 positive results out of 20 replicates) between the methods at 80% power. The determination of smaller differences (< 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-hour incubation time period. Duplicate analysis of approximately 30 org/100mL of EC ATCC 8739 and EC from sewage separated by uncontaminated filter sterilized water were performed. Both the reference method and the Colifast ALARM did not generate positive EC responses for the ATCC 8739 samples after 4 hr (some fluorescence after 24 hr), however, the presence of ATCC 8739 organisms was confirmed by counting on m-Endo plates. The sewage samples were determined accurately by the reference method and the Colifast ALARM. The sewage EC samples were determined to be positive after approximately 11 hours 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 lid to the bottle was tightened and then swirled to dissolve the contents. The bottles were then placed in a water bath that was held between 37-37.5 °C. The vendor instructions called for the TC sample bottles to be incubated for 15-17 hours and the EC sample bottles for 14-16 hours. During this test, the TC samples were incubated for approximately 15.5 hours and 14.5 hours, 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 fluorescent

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 and the appropriate growth media was added to the sample bottle. This sample bottle was incubated for 14 hours 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 the water sample, analyze and read the sample result. Also, the Colifast ALARM method calls for a 14 h analysis, shortening the analysis time from the 48 to 72 hr 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.

Chapter 8
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Appendix

Raw Data from Reference Methods, Colifast ALARM, and Confirmation Analyses

Dilution	Sample No.	SM 9221B/F (2100 series)		Colilert	Colifast		Colifast TC Comfirmation via SM9221B/F (2400 series)		Colifast EC Comfirmation via SM9221B/F (2500 series)	
		TC	EC	EC	TC (2400 series)	EC (2500 series)	TC	EC	TC	EC
A (50 org/100ml)	1	X	X	X	X	O	X	O	X	O
	2	X	X	X	O	O	X	O	X	O
	3	X	O	X	X	O	X	O	X	O
	4	X	O	O	X	O	X	O	X	O
	5	X	X	X	O	O	X	O	X	O
	6	O	O	O	X	O	X	O	X	O
	7	X	O	X	X	O	X	O	X	O
	8	X	O	X	X	X	X	X	X	X
	9	X	X	X	O	O	O	O	X	O
	10	X	X	O	X	X	X	O	X	X
	11	X	O	X	X	O	X	O	X	X
	12	X	X	X	X	O	X	O	X	O
	13	X	O	X	X	X	X	X	X	X
	14	X	X	X	X	O	X	X	X	O
	15	X	X	O	X	O	X	X	X	O
	16	X	O	X	X	O	X	O	X	O
	17	X	O	X	X	O	X	X	X	O
	18	X	X	O	X	O	X	O	X	O
	19	X	O	O	X	O	X	O	X	O
	20	X	O	X	X	O	X	O	X	O
Percent Positive=		95%	45%	70%	85%	15%	95%	25%	100%	20%
B (10 org/100ml)	21	O	O	O	O	O	O	O	O	O
	22	O	O	O	O	O	O	O	O	O
	23	X	O	O	X	O	X	O	O	O
	24	X	X	O	O	O	O	O	X	O
	25	O	O	O	X	O	X	O	O	O
	26	X	O	O	O	O	O	O	O	O
	27	X	O	O	O	O	O	O	X	O
	28	X	O	O	O	O	O	O	O	O
	29	X	O	O	X	O	X	O	X	O
	30	X	O	O	O	O	O	O	O	O
	31	X	X	O	O	O	O	O	X	O
	32	O	O	O	O	O	O	O	O	O
	33	X	O	O	X	O	X	O	O	O
	34	O	O	X	X	O	X	O	X	O
	35	X	O	O	X	O	X	O	X	O
	36	O	O	O	O	O	O	O	O	O
	37	X	O	X	O	O	O	O	X	O
	38	O	O	O	O	O	O	O	X	O
	39	X	X	O	X	O	X	O	O	O
	40	X	O	O	O	O	O	O	O	O
Percent Positive=		65%	15%	10%	35%	0%	35%	0%	40%	0%

Dilution	Sample No.	SM 9221B/F (2100 series)		Colilert	Colifast		Colifast TC Comfirmation via SM9221B/F (2400 series)		Colifast EC Comfirmation via SM9221B/F (2500 series)	
		TC	EC	EC	TC (2400 series)	EC (2500 series)	TC	EC	TC	EC
Percent Positive=		65%	15%	10%	35%	0%	35%	0%	40%	0%
C (5 org/100ml)	41	O	O	O	O	O	O	O	X	X
	42	O	O	O	O	O	O	O	O	O
	43	O	O	O	O	O	X	O	X	O
	44	X	O	O	O	O	X	O	X	O
	45	X	O	O	O	O	O	O	O	O
	46	O	O	O	X	O	X	X	X	O
	47	O	O	O	X	X	X	O	X	X
	48	O	O	O	O	O	O	O	O	O
	49	O	O	X	O	O	O	O	X	O
	50	X	O	X	O	O	O	O	O	O
	51	O	O	X	O	O	O	O	X	O
	52	O	O	O	O	O	O	O	O	O
	53	O	O	O	O	O	O	O	O	O
	54	O	O	O	X	O	X	O	O	O
	55	O	O	O	O	O	X	O	X	O
	56	O	O	O	X	O	O	O	O	O
	57	X	O	O	O	O	O	O	X	O
	58	O	O	O	O	O	O	O	O	O
	59	O	O	O	O	O	O	O	O	O
	60	O	O	O	X	O	X	O	O	O
Precent Positive=		20%	0%	15%	25%	5%	35%	5%	45%	10%
Controls										
Method Blank	64	O	O	O	O	O	O	O	O	O
	69	O	O	O	O	O	O	O	O	O
	72	O	O	O	O	O	O	O	O	O
TC Positive (Ea)	61	X	O	O	X	O	X	O	X	O
	65	X	O	O	X	O	X	O	X	O
	70	X	O	O	X	O	X	O	X	O
Ec Positive (Ec)	62	X	X	X	X	X	X	X	X	X
	67	X	X	X	X	X	X	X	X	X
	71	X	X	X	X	X	X	X	X	X
TC Neg/Ec Neg (Pa)	63	O	O	O	O	O	O	O	O	O
	66	O	O	O	O	O	O	O	O	O
	68	O	O	O	O	O	O	O	O	O
X= Presence										
O= Absence										