September 2010

Environmental Technology Verification Report

PATHOGEN DETECTION SYSTEMS, INC.
AUTOMATED MICROBIOLOGY PLATFORM

Prepared by
Battelle

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

ETV ETV ETV

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ETV Advanced Monitoring Systems Center

PATHOGEN DETECTION SYSTEMS, INC.

AUTOMATED MICROBIOLOGY PLATFORM

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.

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List of Abbreviations

ADQ	audit of data quality
AMP	Automated Microbiology Platform
AMS	Advanced Monitoring Systems
ATCC	American Type Culture Collection
BGLB	brilliant green lactose bile
cm	centimeters
COC	chain of custody
DDW	dechlorinated drinking water
DTU	desktop testing unit
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
LTB	lauryl tryptose broth
MB	method blank
min	minute(s)
mL	milliliter
MUG	4-methyllumbelliferyl-β-D-glucorinide
N	number
n/a	not applicable
NA	nutrient agar
NRMRL	National Risk Management Research Laboratory
org	organisms
PDS	Pathogen Detection Systems
ppm	parts per million
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
SM	Standard Methods
SOP	Standard Operating Procedure
SSDW	spiked, stressed drinking water
SWTP	Southerly Wastewater Treatment Plant
TQAP	Test Quality Assurance Plan
TC	total coliform
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 Automated Microbiology Platform (AMP) by Pathogen Detection Systems, Inc. (PDS), a bench top incubator/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 PDS Automated Microbiology Platform (hereafter referred to as the PDS AMP). The following is a description of the PDS AMP, based on information provided by the vendor.

The PDS AMP is a bench top incubator/analyzer/data logger system for the analysis of TC and EC. It utilizes an enzyme substrate test to simultaneously detect the presence of TC (β-galactosidase enzyme) and EC (β-glucuronidase enzyme). The system consists of single-use cartridges that contain pre-measured reagents and a chemical-optical sensor. A 100 mL water sample is added to the cartridge and then incubated in and analyzed by the PDS Desktop Testing Unit (DTU), which is the major hardware component of the PDS AMP. The PDS DTU is the blue unit shown in Figure 2-1.

Figure 2-1. PDS Desktop Testing Unit

Figure 2-2. Positive (top) and negative (bottom) test results

The enzymes produced by TCs and EC cleave the fluorogenic substrates in the growth media, resulting in the release of fluorescent products. The fluorescent product molecules rapidly accumulate into a proprietary, polymer-based optical sensor embedded in the test cartridge, which is continuously illuminated by an ultraviolet light source. The light emitted by the optical sensor is detected at wavelengths specific to each fluorescent product. Therefore, the presence of TC and EC can be determined simultaneously by a light detector containing a charge-coupled device. Test management software within the computer accompanying the PDS AMP automatically interprets these optical signals continuously throughout the test cycle and when the PDS AMP is operated in continuous mode, 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 computer provided with the PDS AMP and can be downloaded with a universal serial bus (USB) drive for viewing with the PDS AMP software.

In continous mode, the PDS AMP can analyze two samples in 24 hours (h). PDS provided four units for testing, limiting the sample capacity to eight samples per 24 h. The large number of samples required for this verification test exceeded that capacity. Therefore, the PDS AMP was used mostly in manual mode. In manual mode, following the addition of a water sample to the cartridges, the cartridges were incubated in laboratory incubators for the specified time (18 and 24 h before being inserted into the PDS DTU for a 30-second fluorescent measurement. The results were displayed on the screen in the same manner as for the continuous measurements.

The PDS DTU (not including corresponding desktop computer) has dimensions of 20 centimeters (cm) wide × 30 cm deep ×15 cm high (8 inches (in) wide × 16 in deep ×12 in high) and weighs approximately 5 kilograms (11 pounds). The PDS DTU, computer, and all required software costs approximately $10,000. Sample cartridges can be purchased for approximately $10 per cartridge. The PDS DTU is completely 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 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 the presence of 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[2], such that technologies that are not already approved have an opportunity to be tested under a similar set of test conditions.

3.2 Test Overview

This verification test was conducted according to procedures specified in the Test/QA Plan (TQAP) for Verification of Coliform Detection Technologies for Drinking Water[3] and adhered to the quality system defined in the ETV AMS Center Quality Management Plan (QMP)[4].

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, 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 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 with the detection technology being tested.

In this report, results from the PDS AMP were compared to the results obtained from the reference method analyses which were presence/absence methods for TC and EC, specifically, Standard Methods (SM)[5] 9221B (TC) and 9221F (EC). These methods utilize selective and/or chromatogenic liquid growth media to detect TC and EC. The verification test of the PDS AMP was conducted from July 19 through July 27, 2010 at Battelle in Columbus, Ohio with the reference method analyses being performed at Superior Laboratories in Galloway, Ohio (a 20 minute drive from Battelle). Additional testing was performed on August 16–18, 2010 at these facilities. Technology operation and sample handling and analysis were performed according to the vendor's instructions. Both reference method and PDS AMP sample analysis results were reported as presence/absence.

Sample analysis results from the PDS AMP 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 (or specificity) and false negative rate (or sensitivity). 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[1]. 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 1 liter [L]), was collected at 7 a.m. on July 19, 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 July 18 – midnight July 19). The SWTP automated system collects 50 to 100 mL aliquots at approximately 5-minute 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 does not believe that there was 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 ensure 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 5 minutes with cold water and 67 L of DW was 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[6]	n/a	6.9
temperature	°C	calibrated thermometer	SOP GEN.V-013-047[7]	n/a	23
free chlorine	mg/L	HACH Chlorine test kit	HACH Method 8021	n/a	0.80
total chlorine	mg/L	HACH Chlorine test kit	HACH Method 8167	n/a	0.80
total, culturable heterotrophic bacteria	org/100 mL	R2A agar	AOAC's Bacteriological Analytical Manual[8]	8.5×10^8	n/a
TC	org/100 mL	m-Endo	SM 9222B	5.7×10^6	n/a
EC	org/100 mL	NA-MUG	SM 9222G	8.0×10^5	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 PDS AMP was tested with chlorine stressed TC and EC. The chlorination stressing step was started within 4 h from the time Battelle received the sample, or approximately 11 h from the time the last automated sample was collected and 35 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 37 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 10 L 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). The three aliquots were chlorinated for 2.5, 5, and 10 minutes, 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 PDS AMP, the 5-minute chlorine stressing attained a two log reduction in both TC and EC; therefore, after being 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 organism per 100 mL were prepared. EC concentrations are typically 10 times less than the TC concentrations. To ensure that these concentrations would be attained for both TC and EC, a range of concentrations was 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 0.1 org/100 mL, 1 org/100 mL, and 10 org/100 mL. Each dilution was mixed on a stir plate for 5 to 10 minutes, and then 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 per technology), verification testing was initiated. All samples were stored refrigerated during the day of preparation until the analysis was initiated that same day.

In addition to the samples to be used for PDS 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 Standard Operating Procedure (SOP) ENV-ADM-009 for Chain of Custody[9]. The COC form was signed once receipt of all samples was confirmed. Reference method analysis was initiated on the same day as arrival at the laboratory, within 2 h of initiation of the PDS sample analysis.

3.3.2 Sample Analysis

The ability of the PDS AMP to determine the presence of TC and EC was challenged using 20 replicates of the three concentrations of SSDW samples. 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. PDS provided four DTUs to perform testing of the replicate samples shown in Table 3-3. Each of the PDS DTUs contained two sample chambers for incubating and measuring the fluorescence from the sample cartridges. However, as mentioned in Chapter 2, because of the large number of concurrent sample analyses required during this verification test, 66 out of the 72 samples were incubated apart from the PDS DTUs and then inserted into the PDS DTUs at the end of the incubation periods (18 and 24 h) for fluorescent measurement. Six samples were analyzed in continuous detection mode (i.e., they were inserted into the PDS DTU at the start of the incubation and left there for the full 24 h analysis period). All of the samples were assayed by the reference methods and the PDS AMP 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 PDS AMP	Replicate Analyses by SM9221B	Replicate Analyses by SM9221F
Dilution A - 10 org/100 mL	20	20	20
Dilution B - 1 org/100 mL	20	20	20
Dilution C - 0.1 org/100 mL	20	20	20
Method Blank	3	3	3
TC Positive control	3	3	3
EC Positive control	3	3	3
Negative control	3	3	3
Total Replicate Analyses	72	72	72

3.3.2.1 Confirmation of Results

All reference and PDS results were confirmed with more definitive tests to adequately verify the PDS AMP. Confirmation for the SM 9221B and 9221F reference methods, as well as the PDS AMP, is described in detail in the TQAP. In summary, for the PDS AMP analyses, 1 mL of each 100 mL sample resulting from the 24 h incubation during PDS analysis was inoculated into 9 mL of lauryl tryptose broth (LTB) and analyzed using SM 9221 B and F. 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 PDS AMP and SM 9221B and 9221F were confirmed. As an additional, optional confirmation, a complete test for TC was performed for three samples by inoculating MacConkey media and then selecting suspected TC colonies and inoculating into LTB, as described by SM 9221B.

3.3.3 Detection of Additional Concentration Levels in Continuous Operating Mode

An optional component of the ETV test was performed to verify the capability of the PDS AMP to detect EC ATCC 8739 at various concentration levels in continuous operating mode, which

provides positive results as soon as determined by the PDS DTU. Four target inoculations were prepared in DDW that contained approximately 10^4 EC per 100 mL, and then a serial dilution (1:10, 1:100, and 1:1,000) of the stock was prepared to obtain four separate samples for testing (10, 100, 1,000 and 10,000 EC per 100 mL). The data from these tests were intended to identify: 1) whether or not each technology detects the presence of EC and 2) the time required for detection. Triplicate aliquots at each concentration level were analyzed using a quantitative method for EC (SM 9222G – NA-MUG) to confirm the concentration of the samples.

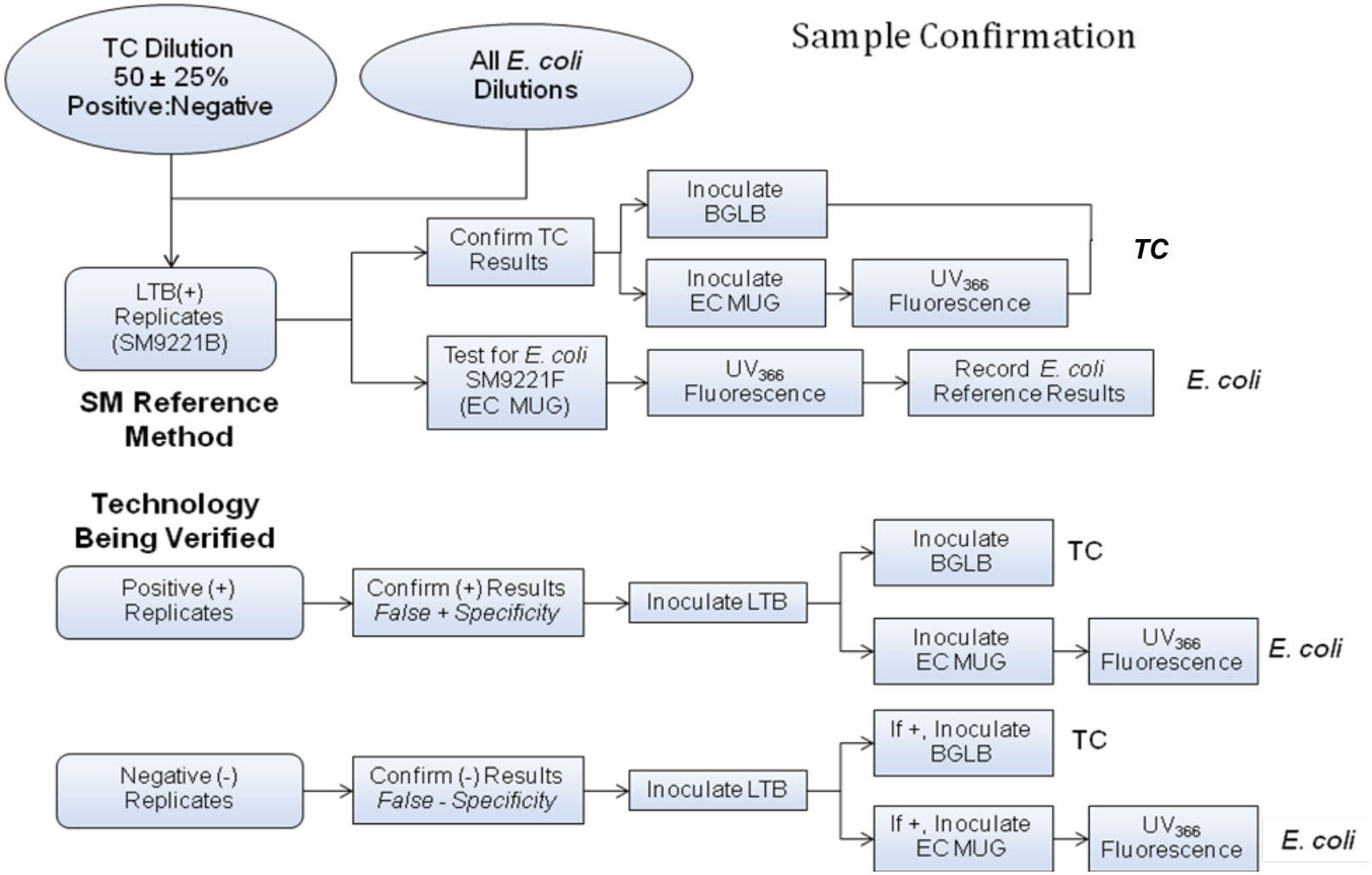

Figure 3-1. Flowchart describing confirmation analyses for both the PDS AMP and SM9221B and F

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 was one deviation from the TQAP (described in Section 3.3.1.1 involving 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. This deviation was judged by the Battelle Verification Test Coordinator to not result in any adverse impacts on the quality of the data generated. The deviation was 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 (MBs), 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 PDS AMP as well as the reference method were negative, indicating the absence of TC and EC.

Three positive and negative control samples were also analyzed using each method. 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 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 PDS AMP and reference methods at relatively high concentrations. The control cultures were not enumerated, but were estimated to be approximately 10^5 to 10^6 org/100 mL based on the degree of turbidity observed in the sample.

All three TC positive controls were determined to be positive using the reference method and the PDS AMP (and confirmed to be positive during the confirmation analysis). In addition, all three EC positive controls were determined to be positive (for both TC and EC*)* using the reference method and the PDS AMP (and confirmed to be positive during the confirmation analysis). One out of three TC negative control samples was found to be positive (the other two were properly negative) for TC during the PDS AMP and reference analysis; however, this sample was also found to be positive during the PDS AMP confirmation analysis. Apparently there was TC contamination in the sample container causing positive results. However, there was no other indication of contamination throughout the rest of the test. All of the other negative control samples generated expected negative results and the method blank samples all produced negative results. While the organism was not isolated and identified, it seems as though this was an isolated instance of TC contamination of the sample container or of the negative control culture and did not adversely impact the results of the test.

4.2 Audits

Two types of audits were performed during the verification test; a technical systems audit (TSA) of the verification test procedures, and an audit of data quality (ADQ). Audit procedures for the TSA and the ADQ 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. 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 received a satisfactory response.

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

- Documentation of reference laboratory microbiology training;
- Generation of a deviation to more accurately describe the collection of the sewage water sample;
- Clarification and additional detail to document the sewage sample collection on the custody form.

A TSA report was prepared and distributed to the Verification Test Coordinator, the Battelle AMS Center Manager, the EPA AMS Center Project Officer, and the EPA AMS Center Quality Manager.

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, ADQs were conducted on August 4-6, 2010 and August 24-26, 2010. During the audits, laboratory data generated at the reference laboratory, Superior Laboratories, Inc. and data generated by the PDS AMP were reviewed and verified for completeness, accuracy and traceability. Because this verification testing could potentially be referenced by the Office of Water, it was decided to establish the testing as a Quality Category II, requiring a QA review of 25% of the test data, and a minimum of three peer-reviewers. 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.

Two (2) findings and three (3) observations were identified during the ADQs. The two findings involved documentation of failed QC samples and spreadsheet version control. Findings and observations from the audits were addressed through the following actions.

- In one instance, a trypticase soy broth (TSB) positive control tested negative for EC. Upon review of all available documentation, the negative result was unable to be explained as it was confirmed that the lot of TSB had been used previously to successfully grow EC. It is suspected that the control was inadequately inoculated with EC at the time. There was no adverse impact as TSB was only used in the test as the growth medium to verify the sterility of the PBS used to serial dilute and enumerate the filtered sewage sample.
- Two transcription errors from the original data sheet into a spreadsheet were corrected and a spreadsheet formula pertaining to the percent positive results was also corrected.
- Documentation of the reference laboratory reagent controls was added to the project file.

None of these had an adverse impact on the test results and all findings received a satisfactory response. A data audit report was prepared, and a copy was distributed to the EPA AMS Center Quality Manager.

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 PDS AMP were evaluated when assessing comparability. During this test, true positives (TPs) were those positive results from the PDS AMP that were confirmed, and FPs were those positive results from the PDS AMP that were not confirmed by the reference method. Conversely, true negative (TN) results were those negative results that were confirmed as negative, and FN results were those negative results that were shown to be positive by the confirmatory method. Performance of the PDS AMP 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, FP rates, and FN rates as percentages for the two methods were calculated as follows:

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

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

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

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

5.2 Method Comparability

In order to assess whether the proportion of positive and negative samples were significantly different between the PDS AMP 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. If the calculated chi-square value is less than the critical value, the sample results between the two methods are not significantly different (95% confidence, alpha = 0.05, p-value > 0.05). If the chi-square value is greater than the critical value (p-value ≤ 0.05), the results between the two methods are significantly different, and it is concluded that there is a difference between the two methods.

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. 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 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 (five positive results and 15 negative results), then the technology being tested would be required to be 65% positive (13 positives and seven negative results) to have a significant difference. PDS AMP results with a higher 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 PDS AMP results that were 11% or less positive or negative (less than two positives and 18 negatives or more than 18 positives and two negatives). Finally, if the reference method was 65% positive, then a significant difference could be determined with at most a 32% positive result. The PDS AMP 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 approximately a 30% to 40% change in positive results. The power analysis revealed that differences of 5% or 10% of 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 PDS AMP with those generated by the presence/absence result from the reference methods. The qualitative evaluation was performed to document the operational aspects of the PDS AMP 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 PDS AMP, and the confirmation analyses are provided in the Appendix.

6.1 TC Data

The positive TC test results for the PDS AMP and reference method (SM 9221B) are presented in Table 6-1. One of the three dilutions yielded the target 50 ± 25% split in responses for the reference method. However, a second dilution generated results that were similar to the targeted range (85% positive, 15% negative). Therefore, results for both of these dilutions (Dilutions A and B) are reported. Table 6-1 summarizes the positive TC test results for the PDS AMP incubated for 18 and 24 h.

Table 6-1. TC Positive Results

	PDS 18 h		PDS 24 h		SM 9221B	
Dilution	N	% of total samples	N	% of total samples	N	% of total samples
A (10 org/100 mL)	13	65%	19	95%	17	85%
B (1 org/100 mL)	2	10%	5	25%	5	25%

N - Number

Tables 6-2 and 6-3 summarize the TP (confirmed) and TN (confirmed) TC results for the PDS AMP (18 and 24 hour incubations). The reference method data are also presented.

Table 6-2. TC Data Summary - Positives

	PDS 18 h			PDS 24 h			SM 9221B
Dilution	N	Confirmed TP	Difference (FP)	N	Confirmed TP	Difference (FP)	N
A (10 org/100 mL)	13	13	0	19	17	2	17
B (1 org/100 mL)	2	2	0	5	4	1	5

Table 6-3. TC Data Summary - Negatives

	PDS 18 h			PDS 24 h			SM 9221B
Dilution	N	Confirmed TN	Difference (FN)	N	Confirmed TN	Difference (FN)	N
A (10 org/100 mL)	7	3	4	1	1	0	3
B (1 org/100 mL)	18	14	4	15	13	2	15

The sensitivity, specificity, FP, and FN rates for the PDS AMP 18 hour and 24 hour TC results were determined as described in Section 5.1 and are presented in Table 6-4.

Table 6-4. TC Data Summary - Confirmations

Incubation Time (h)	18 h	24 h
Sensitivity	65%	91%
Specificity	100%	82%
False Positive	0%	18%
False Negative	35%	9%

6.2 EC Data

Table 6-5 summarizes the positive EC test results for the PDS AMP incubated for 18 and 24 h according to the manufacturer's directions. The results for the reference method (SM 9221F) are also presented.

Table 6-5. EC Positives

	PDS 18 h		PDS 24 h		SM 9221F	
Dilution	N	% of total samples	N	% of total samples	N	% of total samples
A (10 org/100 mL)	8	40%	8	40%	6	30%

Tables 6-6 and 6-7 summarize the confirmed TP and TN EC results for the PDS AMP (18 and 24 hour incubations). The reference method data are also presented.

Table 6-6. EC Summary – Positives

	PDS 18 h			PDS 24 h			SM 9221F
Dilution	N	Confirmed TP	Difference (FP)	N	Confirmed TP	Difference (FP)	N
A (10 org/100 mL)	8	8	0	8	8	0	6

Table 6-7. EC Summary – Negatives

	PDS 18 h			PDS 24 h			SM 9221F
Dilution	N	Confirmed TN	Difference (FN)	N	Confirmed TN	Difference (FN)	N
A (10 org/100 mL)	12	8	4	12	8	4	14

The sensitivity, specificity, FP, and FN rates for the PDS AMP 18 hour and 24 hour EC results were determined as described in Section 5.1 and are presented in Table 6-8.

Table 6-8. EC Data Summary – Confirmations

Incubation Time (h)	18 h	24 h
Sensitivity	67%	67%
Specificity	100%	100%
False Positive	0%	0%
False Negative	33%	33%

6.3 Method Comparability

Tables 6-9 and 6-10 show the results from the chi-square test for independence that was performed to compare the TC results from the PDS AMP for each incubation time period against the reference method (SM 9221B). For TC, data from each of the two dilutions were tested separately and together. The chi-square value for each of the TC dilutions, as well as the additive chi-square value, was less than the critical limits; therefore, the chi-square test did not detect any differences between the results of the PDS AMP and the reference method at 18 or 24 h. The calculated p-values were greater than 0.05, indicating that the data did not show a statistically significant difference between the two methods for the detection of TCs.

Table 6-9. TCs – 18 h

Dilution	PDS AMP		SM9221B		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (10 org/100 mL)	13	7	17	3	2.13	1	0.144	3.841
B (1 org/100 mL)	2	18	5	15	1.56	1	0.212	3.841
Additive Result					3.69	2	0.164	5.991

Table 6-10. TCs – 24 h

Dilution	PDS AMP		SM9221B		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (10 org/100 mL)	19	1	17	3	1.11	1	0.292	3.841
B (1 org/100 mL)	5	15	5	15	0.00	1	1.00	3.841
Additive Result					1.11	2	0.574	5.991

These results are consistent with the power analysis performed before testing and described in Section 5.2.

For TC, the reference method generated 85% positive results for Dilution A. While the power analysis was only performed for reference method proportions between 25% and 75%, when the reference method was 75% positive (15 positive and five negative), the technology being tested was required to be approximately 65% negative (seven positive and 13 negative) to be considered different from the reference method. Had the power analysis been applied to the reference method being 85% positive, a result that was significantly different from the reference would be similar in proportion, but slightly less negative (possibly eight or nine positive and 12 or 11 negative). Given the Dilution A result of 13 positive and seven negative at 18 h and 19 positive and one negative at 24 h, neither result was more than 50% negative, confirming that the chi-square result was consistent with the power analysis. It is possible that smaller differences between the reference method and the PDS AMP could be determined if more replicates were included in the experimental design.

For Dilution B, the TC reference method results were 25% positive. This was the same proportion as negative Dilution A. Therefore, the evaluation of the results in the context of the power analysis was the same as for Dilution A, only with the opposite sign. Since the reference method was 25% positive, the power analysis showed that a result with approximately 65% positive (13 positive and seven negative) would be required to exhibit a significant difference. The PDS results for Dilution B were 10% positive (two positive and 18 negative) after 18 h, slightly less positive than the reference method and not close to the difference required to determine a significant difference. The result after 24 h was an exact match to the reference method. Both of these results confirmed that the chi-square result was consistent with the power analysis.

Tables 6-11 and 6-12 show the results from the chi-square test for independence that was performed to compare the EC results from the PDS AMP for each incubation time period against the reference method (SM 9221F). For EC, 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 PDS AMP and the reference method at 18 or 24 h. 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.

Table 6-11. EC – 18 h

Dilution	PDS AMP		SM9221F		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (10 org/100 mL)	8	12	6	14	0.440	1	0.507	3.841
Additive Result					0.440	1	0.507	3.841

Table 6-12. EC – 24 h

Dilution	PDS AMP		SM9221F		Chi-Square	Degrees of freedom	p-Value	Critical Limits (p=0.05)
	+	-	+	-				
A (10 org/100 mL)	8	12	6	14	0.440	1	0.507	3.841
Additive Result					0.440	1	0.507	3.841

As was the case for TC, the EC results are consistent with the power analysis performed before testing. The proportion of positive results from the reference method was 30% (six positive and 14 negative). According to the power analysis, approximately a 75% positive result (15 positive and five negative) would be required from the PDS AMP for a significant difference to be determined between the reference method and the PDS AMP. The PDS AMP result after both 18 h and 24 h was 40% positive (eight positive and 12 negative), slightly more positive than the reference method and not close to the difference required to determine a significant difference, confirming the chi-square results as being consistent with the power analysis. The determination of significant differences in EC results was also limited by the number of replicates as was described above.

6.4 Detection of Additional Concentration Levels in Continuous Operating Mode

The objective of this component of the testing was to verify the PDS AMP capability of reporting analysis results as soon as determined by the PDS DTU rather than waiting for the end of an incubation time period such as 18 or 24 h. Table 6-13 gives the results for the analysis of various concentration of EC ATCC 8739 including the result provided and the time of result. In general, the PDS AMP did not generate positive EC responses except for two of the 10,000 EC/100 mL samples. However, all of the samples were reported as positive for TC. Four replicate samples of each concentration were analyzed and the TC positive results were reported

between 14 and 16 h for 10 EC /100 mL, 13and 15 h for 100 EC /100 mL, 11.5 and 13.5 h for 1,000 EC /100 mL, and 10 and 11.5 h for 10,000 EC /100 mL. The two positive EC results were reported in approximately 23 h, just before the end of the 24 h analysis period.

Table 6-13. Results of Analysis of Additional Concentrations in Continuous Operation Mode

EC Conc. (org/100mL)	TC	Incubation Time TC Detected (h:min)	EC	Incubation Time EC Detected (h:min)
10	X	15:45	O	ND
	X	15:08	O	ND
	X	14:48	O	ND
	X	14:02	O	ND
100	X	14:53	O	ND
	X	14:43	O	ND
	X	13:22	O	ND
	X	13:02	O	ND
1,000	X	13:17	O	ND
	X	12:46	O	ND
	X	12:07	O	ND
	X	11:48	O	ND
10,000	X	10:55	O	ND
	X	10:18	O	ND
	X	11:21	X	23:16
	X	10:34	X	22:59

X=Presence; O= Absence

6.5 Operational Factors

The verification staff found that the PDS AMP was easy to use. A PDS representative came to Battelle to set up the equipment and train the verification staff in the operation of the PDS AMP. The PDS AMP was set up by plugging the PDS DTU and desktop computer into standard 110 volt power and powering up. For operation in continuous mode, no special laboratory facilities were required. In manual mode, laboratory incubators were required. Following an approximately 30-minute training session, the operators (consisting of Battelle microbiology technicians) were comfortable operating the PDS AMP without assistance.

Prior to use for water samples, PDS required the analysis of three control cartridges that do not contain any liquid, but contain the same polymer bottom (as the standard sample cartridges) that fluoresces at the proper wavelengths to indicate the presence of TC only with one cartridge, TC and EC with a second cartridge, and neither with the third cartridge. Once these control

cartridges had been analyzed and reported the proper results, the PDS DTUs were ready for the analysis of samples.

As previously described, the PDS AMP was operated in both manual and continuous measurement mode for the simultaneous measurement of TC and EC using the same 100 mL cartridge. In manual mode, 100 mL of the water sample was dispensed into each cartridge and the cartridge was snapped firmly shut and swirled to dissolve the contents. The cartridges were then placed in an incubator that was held between 35 and 36 °C for 24 h. After 18 h, the cartridges were removed from the incubator and inserted into the PDS DTU for an initial measurement. The cartridges were measured two at a time by clicking on a "start" button on the computer screen. The measurement of two samples took approximately 30 seconds and the cartridges were immediately returned to the incubator for the remaining 6 h to complete the 24 h incubation. The measurement step was repeated after the 24 h incubation was complete.

In continuous operation mode, the samples were loaded into the cartridges in an identical fashion and the 24 h incubation/analysis was started. The samples (two at a time) were incubated within the PDS DTU and results were reported on the screen as soon as the PDS AMP was able to make a conclusive determination of TC and/or EC based on the fluorescence measurement. A positive result could have been reported at any point during the 24 h analysis, while a negative result would not occur until the end of the 24 h incubation. During the continuous measure, a countdown timer appeared on the computer screen to indicate the time remaining for the analysis. The continuous operation mode eliminates the need for a technician to be present to read the sample result. Also, the PDS AMP method calls for a 18 h or 24 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.

During the measurement step in both modes, the result of each measurement was displayed on the screen and the operator recorded the result on a sample data sheet. Each result could also be downloaded for review and viewed on a computer containing the PDS AMP software, but the results from a group of samples could not be exported as a spreadsheet. The PDS DTU (not including corresponding desktop computer) has dimensions of 20 cm wide × 30 cm deep ×15 cm high (8 in wide × 16 in deep ×12 in high) and weighs approximately 5 kilograms (11 pounds). The PDS DTU, computer, and all required software costs approximately $10,000. Sample cartridges can be purchased for approximately $10 per cartridge.

Chapter 7
Performance Summary

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 PDS AMP 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 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 method were compared with the PDS AMP. The results of the verification of the PDS AMP are summarized below:

Positive Results. Table 7-1 summarizes the positive TC test results for the PDS AMP incubated for 18 and 24 h.

Table 7-1. Results Summary for Positive PDS AMP Results for TC and EC

TC or EC	Dilution	PDS 18 h		PDS 24 h		SM 9221B/F	
		N	% of total samples	N	% of total samples	N	% of total samples
TC	A (10 org/100 mL)	13	65%	19	95%	17	85%
	B (1 org/100 mL)	2	10%	5	25%	5	25%
EC	A (10 org/100 mL)	8	40%	8	40%	6	30%

Specificity, Sensitivity, FP rate, and FN rate. Table 7-2 summarizes the specificity, sensitivity, FP rate, and FN rate for TC and EC for 18 and 24 h incubations. 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 PDS AMP for 18 and 24 h Incubation Times

	TC		EC	
	18	**24**	**18**	**24**
Sensitivity	65%	91%	67%	67%
Specificity	100%	82%	100%	100%
False Positive Rate	0%	18%	0%	0%
False Negative Rate	35%	9%	33%	33%

Comparability. In another approach of comparison, a chi-square test for independence was performed to compare the PDS AMP for each incubation time period against the reference methods (SM 9221B and F). For the EC and TC results, data from each dilution was tested separately and for the TC only, two dilution levels were tested together. The chi-square value for the EC solution and each of the TC dilutions, as well as the additive chi-square value, was less than the critical limit in each case; therefore, for EC and TC, the chi-square test did not detect any differences between the results of the PDS AMP and the reference method at 18 or 24 h. 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 or TC at the 95% confidence interval. These results were consistent with the power analysis performed before testing and described in Section 5.2. This power analysis showed the number of replicate samples required for significant differences at a minimum of 80% power. It showed that the smallest difference that is able to be determined with 20 replicates was approximately a 30% to 40% change in positive results for each dilution. The power analysis also revealed that differences of 5% or 10% of positive results could be determined, but between 150 and 1,250 replicates may be required.

Additional Concentrations in Continuous Operation. The objective of this component of the testing was to verify the PDS AMP capability of reporting analysis results as soon as determined by the PDS AMP rather than waiting for the end of an incubation time period such as 18 or 24 h. Four concentrations of EC ATCC 8739 (10, 100, 1,000, and 10,000 EC/100 mL) were analyzed four times each. The PDS AMP did not generate positive EC responses except for two of the 10,000 EC/100 mL samples. However, all of the samples were reported as positive for TC in an average time of approximately 13 hours. An observation was made that the amount of time until detection for the TC samples decreased with each increasing concentration level.

Operational Factors. The PDS AMP was operated in both manual and continuous measurement mode for the simultaneous measurement of TC and EC using the same 100 mL cartridge. In manual mode, 100 mL of the water sample was dispensed into each cartridge and the cartridge was snapped firmly shut and swirled to dissolve the contents. The cartridges were then placed in an incubator that was held between 35 and 36 °C for 24 h. After 18 h, the cartridges were removed from the incubator and inserted into the PDS DTU for an initial measurement. The cartridges were measured two at a time by clicking on a “start” button on the computer screen. The measurement of two samples took approximately 30 seconds and the cartridges were immediately returned to the incubator for the remaining 6 h to complete the 24 h incubation. The measurement step was repeated after the 24 h incubation was complete.

In continuous operation mode, the samples were loaded into the cartridges in an identical fashion and the 24 h incubation/analysis was started. The samples (two at a time) were incubated within the PDS DTU and results were reported on the screen as soon as the PDS AMP was able to make a conclusive determination of TC and/or EC based on the fluorescence measurement. The continuous operation mode eliminates the need for a technician to be present to read the sample result. Also, the PDS AMP method calls for a 18 h or 24 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.

During the measurement step in both modes, the result of each measurement was displayed on the screen and the operator recorded the result on a sample data sheet. Each result could also be downloaded for review and viewed on a computer containing the PDS AMP software, but the results from a group of samples could not be exported as a spreadsheet.

The PDS DTU (not including corresponding desktop computer) has dimensions of 20 cm wide × 30 cm deep ×15 cm high (8 in wide × 16 in deep ×12 in high) and weighs approximately 5 kilograms (11 pounds). The PDS DTU and computer and all required software costs approximately $10,000. Sample cartridges can be purchased for approximately $10 per cartridge.

Chapter 8
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Appendix

Raw Data from Reference Methods, PDS AMP, and Confirmation Analyses

Dilution	Sample No.	SM 9221B/F		PDS 18H		PDS 24H		PDS Confirmation via SM9221B/F	
		TC	EC	TC	EC	TC	EC	TC	EC
A (10 org/100mL)	1	X	O	X	X	X	X	X	X
	2	X	X	X	O	X	O	X	X
	3	X	O	X	X	X	X	X	X
	4	X	O	O	O	O	O	O	O
	5	X	O	X	O	X	O	X	O
	6	X	O	O	O	X	O	X	O
	7	X	O	X	X	X	X	X	X
	8	X	O	O	O	X	O	X	X
	9	X	O	O	O	X	O	X	O
	10	X	X	O	O	X	O	O	O
	11	X	X	X	X	X	X	X	X
	12	X	X	X	O	X	O	X	X
	13	X	O	X	X	X	X	X	X
	14	X	X	X	X	X	X	X	X
	15	O	O	X	O	X	O	X	X
	16	O	O	X	O	X	O	X	O
	17	O	O	X	X	X	X	X	X
	18	X	O	O	O	X	O	O	O
	19	X	X	X	O	X	O	X	O
	20	X	O	O	X	X	X	X	X
Percent Positive=		85%	30%	65%	40%	95%	40%	85%	60%

X= Presence

O= Absence

Dilution	Sample No.	SM 9221B/F		PDS 18H		PDS 24H		PDS Confirmation via SM9221B/F	
		TC	EC	TC	EC	TC	EC	TC	EC
B (1 org/100mL)	21	O	O	O	O	O	O	O	O
	22	X	O	O	O	O	O	O	O
	23	X	O	O	O	O	O	O	O
	24	O	O	O	O	O	O	X	O
	25	X	O	O	O	O	O	O	O
	26	O	O	O	O	X	O	O	O
	27	X	O	X	O	X	O	X	O
	28	O	O	O	O	O	O	O	O
	29	O	O	O	O	O	O	X	O
	30	O	O	X	O	X	O	X	X
	31	O	O	O	O	O	O	O	O
	32	O	O	O	O	X	O	X	O
	33	O	O	O	O	X	O	X	O
	34	O	O	O	O	O	O	O	O
	35	O	O	O	O	O	O	O	O
	36	O	O	O	O	O	O	O	O
	37	O	O	O	O	O	O	O	O
	38	O	O	O	O	O	O	O	O
	39	O	O	O	O	O	O	O	O
	40	X	O	O	O	O	O	O	O
Percent Positive=		25%	0%	10%	0%	25%	0%	30%	5%

X= Presence

O= Absence

Dilution	Sample No.	SM 9221B/F		PDS 18H		PDS 24H		PDS Confirmation via SM9221B/F	
		TC	EC	TC	EC	TC	EC	TC	EC
C (0.1 org/100mL)	41	O	O	O	O	O	O	O	O
	42	O	O	O	O	O	O	O	O
	43	O	O	O	O	O	O	O	O
	44	O	O	O	O	O	O	O	O
	45	O	O	O	O	O	O	O	O
	46	O	O	O	O	O	O	O	O
	47	O	O	O	O	O	O	O	O
	48	O	O	O	O	O	O	O	O
	49	O	O	O	O	O	O	O	O
	50	O	O	O	O	O	O	O	O
	51	O	O	O	O	O	O	O	O
	52	O	O	O	O	O	O	O	O
	53	O	O	O	O	O	O	O	O
	54	O	O	O	O	O	O	O	O
	55	O	O	O	O	O	O	O	O
	56	O	O	O	O	O	O	O	O
	57	O	O	O	O	O	O	O	O
	58	O	O	O	O	O	O	O	O
	59	O	O	O	O	O	O	O	O
	60	O	O	O	O	O	O	O	O
Percent Positive=		0%	0%	0%	0%	0%	0%	0%	0%
Controls									
Method Blank	61	O	O	O	O	O	O	O	O
	62	O	O	O	O	O	O	O	O
	63	O	O	O	O	O	O	O	O
TC Positive (Ea)	64	X	O	X	O	X	O	X	O
	65	X	O	X	O	X	O	X	O
	66	X	O	X	O	X	O	X	O
EC Positive (EC)	67	X	X	X	X	X	X	X	X
	68	X	X	X	X	X	X	X	X
	69	X	X	X	X	X	X	X	X
TC Neg/EC Neg (Pa)	70	O	O	O	O	O	O	O	O
	71	O	O	O	O	O	O	O	O
	72	O	O	X	O	X	O	X	O

X= Presence

O= Absence